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PATENT TRADEMARK OFFICE

CHAPTER II

TRANSMITTAL LETTER TO THE UNITED STATES ELECTED OFFICE (EO/US)

(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

PCT/SG98/00045	19 JUNE 1998	19 JUNE 1998
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED

A VACCINE-INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF

TITLE OF INVENTION

1. Chong Jin OON, 2. Gek Keow LIM, 3. Ai Lin LEONG, 4. Yi ZHAO, 5. Wei Ning CHEN

APPLICANT(S)

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231
ATTENTION: EO/US

NOTE: The completion of those filing requirements that can be made at a time later than 30 months from the priority date

CERTIFICATION UNDER 37 C.F.R. 1.10*
*(Express Mail label number is **mandatory**.)*
(Express Mail certification is optional.)

I hereby certify that this correspondence and the documents referred to as attached therein are being deposited with the United States Postal Service on this date DECEMBER 13, 2000, in an envelope as "Express Mail Post Office to Addressee," Mailing Label Number EE784103258US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

SHARON CHU
(type or print name of person mailing paper)
Sharon Chu
Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

***WARNING:** *Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

EE784103258US

results from the Commissioner exercising his judgment under the authority granted under 35 USC 371(d). The filing receipt will show the actual date of receipt of the last item completing the entry into the national phase. See 37 C.F.R. §1.491 which states: "An international application enters the national state when the applicant has filed the documents and fees required by 35 USC 371(c) within the periods set forth in § 1.494 and § 1.495."

WARNING: *Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. §1.10 must be used (since international application papers are not covered by an ordinary certificate of mailing - See 37 C.F.R. §1.8.*

NOTE: Documents and fees must be clearly identified as a submission to enter the national state under 35 USC 371 otherwise the submission will be considered as being made under 35 USC 111. 37 C.F.R. § 1.494(f).

1. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. 371:

   a. [X] This express request to immediately begin national examination procedures (35 U.S.C. 371(f)).

   b. [X] The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees (37 C.F.R. § 1.492) as indicated below:

i. [X] A check in the amount of $860.00 to cover the above fees is enclosed.
ii. [ ] Please charge Account No. _____ in the amount of $ _____.
A duplicate copy of this sheet is enclosed.

**WARNING: *"To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: * * * (2) the basic national fee (see § 1.492(a)). The 30-month time limit may not be extended." 37 C.F.R. § 1.495(b).*

WARNING: *If the translation of the international application and/or the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 C.F.R. § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 apply to the period which is set. Notice of Jan. 3, 1993, 1147 O.G. 29 to 40.*

3. [X] A copy of the International application as filed (35 U.S.C. 371(c)(2)):

NOTE: *Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date." Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.*

a. [ ] is transmitted herewith.
b. [ ] is not required, as the application was filed with the United States Receiving Office.
c. [X] has been transmitted
i. [X] by the International Bureau.
Date of mailing of the application (from form PCT/IB/308): DECEMBER 23, 1999.
ii. [ ] by applicant on _____.
Date

4. [X] A translation of the International application into the English language (35 U.S.C. 371(c)(2)):
a. [ ] is transmitted herewith.
b. [X] is not required as the application was filed in English.
c. [ ] was previously transmitted by applicant on _____.
Date
d. [ ] will follow.

5. [X] Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. 371(c)(3)):

NOTE: The Notice of January 7, 1993 points out that 37 C.F.R. § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that: "The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1.121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 36.

a. [ ] are transmitted herewith.
b. [ ] have been transmitted
   i. [ ] by the International Bureau.
   Date of mailing of the amendment (from form PCT/IB/308): _____.
   ii. [ ] by applicant on _____.
   Date
c. [X] have not been transmitted as
   i. [X] applicant chose not to make amendments under PCT Article 19.
   Date of mailing of Search Report (from form PCT/ISA/210): MARCH 17, 1999.
   ii. [ ] the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. [X] A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. 371(c)(3)):
a. [ ] is transmitted herewith.
b. [ ] is not required as the amendments were made in the English language.
c. [X] has not been transmitted for reasons indicated at point 5(c) above.

7. [X] A copy of the international examination report (PCT/IPEA/409)
[X] is transmitted herewith.
[ ] is not required as the application was filed with the United States Receiving Office.

8. [X] Annex(es) to the international preliminary examination report
a. [X] is/are transmitted herewith.
b. [ ] is/are not required as the application was filed with the United States Receiving Office.

9. [X] A translation of the annexes to the international preliminary examination report
a. [ ] is transmitted herewith.
b. [X] is not required as the annexes are in the English language.

10. [X] An oath or declaration of the inventor (35 U.S.C. 371(c)(4)) complying with 35 U.S.C. 115
    a. [ ] was previously submitted by applicant on _____. Date
    b. [ ] is submitted herewith, and such oath or declaration
        i. [ ] is attached to the application.
        ii. [ ] identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. 1.70.
    c. [X] will follow.

Other document(s) or information included:

11. [X] An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):
    a. [X] is transmitted herewith.
    b. [ ] has been transmitted by the International Bureau.
    Date of mailing (from form PCT/IB/308): _____.
    c. [ ] is not required, as the application was searched by the United States International Searching Authority.
    d. [ ] will be transmitted promptly upon request.
    e. [ ] has been submitted by applicant on _____. Date

12. [X] An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98:
    a. [X] is transmitted herewith.
    Also transmitted herewith is/are:
    [X] Form PTO-1449 (PTO/SB/08A and 08B).
    [X] Copies of citations listed.
    b. [ ] will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. 371(c).
    c. [ ] was previously submitted by applicant on _____. Date

13. [ ] An assignment document is transmitted herewith for recording.

A separate [ ] "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or [ ] FORM PTO 1595 is also attached.

_____
_____
_____

14. [X] Additional documents:
    a. [X] Copy of request (PCT/RO/101)
    b. [X] International Publication No. WO 99/66047
        i. [X] Specification, claims and drawing
        ii. [ ] Front page only
    c. [ ] Preliminary amendment (37 C.F.R. § 1.121)
    d. [X] Other

    FORM PCT/RO/146 DATED JUNE 27, 1998; FORM PCT/RO/106; FORM PCT/RO/105; FORM PCT/IB/301; FORM PCT/RO/146 DATED NOVEMBER 6, 1998; FORM PCT/IB/306; FORM PCT/IB/308; FORM PCT/IPEA/401DATED JANUARY 18, 2000; FORM PCT/IB/332; MARCH 18, 2000 LETTER TO EPO; MARCH 18, 2000 LETTER TO WIPO; FORM PCT/IPEA/408; RESPONSE TO WRITTEN OPINION

15. [X] The above checked items are being transmitted
    a. [X] before 30 months from any claimed priority date.
    b. [ ] after 30 months.

16. [ ] Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on _____, namely:

_____
_____
_____

AUTHORIZATION TO CHARGE ADDITIONAL FEES

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges if extra claims are authorized.*

NOTE: "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

NOTE: "Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

[X] The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 12-0425 .

[X] 37 C.F.R. 1.492(a)(1), (2), (3), and (4) (filing fees)

WARNING: *Because failure to pay the national fee within 30 months without extension (37 C.F.R. § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.*

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Chong Jin OON, et al.

Serial No.: 09/719,533 Group No.: --

Filed: December 13, 2000 Examiner: --

For: A VACCINE INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF

Attorney Docket No.: U 013108-9

Commissioner of Patents and Trademarks

Washington, D.C. 20231

SIRS:

PRELIMINARY AMENDMENT

Please amend the above identified application as follows:

IN THE CLAIMS

Please amend the following claims:

1. (Amended) An isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) and deposited under Accession Nos. P97121504, P97121505 and P97121506 with the European Collection of Cell Culture on 15th December 1997.

14. (Amended) The vector of claim 12, wherein the vector comprises viral DNA.

CERTIFICATE UNDER 37 1.10

I hereby certify that this paper is being deposited with the United States Postal Service on this date July 10, 2001 in an envelope as "EXPRESS MAIL POST OFFICE TO ADDRESSEE" Mailing Label Number EL728213866US addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231

JENNIFER RASHKIN
(Type or print name of person mailing paper)

(Signature of person mailing paper)

NOTE: Each paper or fee referred to as enclosed herein has the number of the "EXPRESS MAIL" mailing label place thereon prior to mailing 37 CFR 1.16(b).

21. (Amended) A purified polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121504, P97121505 and P97121506 with the European Collection of Cell Culture on 15th December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.

22. (Amended) A purified polypeptide obtained from a method which comprises:

(a) introducing a vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121504, P97121505 and P97121506 with the European Collection of Cell Culture on 15th December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine and operatively linked to a promoter of RNA transcription into a suitable host cell;

(b) culturing the resulting host cell so as to produce the polypeptide;

(c) recovering the polypeptide produced in step (b); and

(d) purifying the polypeptide so recovered.

24. (Amended) A purified peptide obtained from a method which comprises:

(a) introducing a vector comprising an isolated nuclei acid encoding a peptide which comprises at least a portion of a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121504, P97121505 and P97121506 with the European Collection of Cell Culture on 15th December 1997 wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1 into a suitable host cell;

(b) culturing the resulting host cell so as to produce the polypeptide;

(c) recovering the polypeptide produced in step (b); and

(d) purifying the polypeptide so recovered.

33. (Amended) The antibodies obtained in claim 27.

37. (Amended)A method for use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such method comprises

(a) obtaining an appropriate nucleic acid sample from the subject; and

(b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine.

38. (Amended) A method for use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such method comprises

(a) obtaining an appropriate nucleic acid sample from the subject; and

(b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface

antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;

(ii) isolating the complex so formed; and

(iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

39. (Amended) The method of claim 37, wherein the nucleic acid sample in step

(a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and

(ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from

the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, wherein the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

40. (Amended) The method of claim 37, wherein the determining of step (b) comprises:

(i) amplifying the nucleic acid present in the sample of step (a); and

(ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

41. (Amended) A method of use of antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such method comprises:

(a) obtaining an appropriate sample from the subject; and

(b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 so as to determine whether a subject is infected.

42. (Amended) The method of claim 37, wherein the isolated nucleic acid,

oligonucleotide, or antibody is labeled with a detectable marker.

43. (Amended) The method of claim 42, wherein the detectable marker is a radioactive isotope, a fluorophor, or an enzyme.

44. (Amended) The method of claim 37, wherein the sample comprises blood, tissue, or sera.

51. (Amended) A method comprising administering the composition of claim 47 for treating a subject infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).

52. (Amended) A method comprising administering the composition of claim 49 for treating a subject infected with a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).

53. (Amended) A method comprising administering the composition of claim 47 for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject.

54. (Amended) A method comprising administering the composition of claim 50 for preventing infection with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject.

57. (Amended) A method for use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein said method comprises:

(a) obtaining an appropriate nucleic acid sample from the subject; and

(b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid

sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.

58. (Amended) The method of claim 57, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) contacting the mRNA with an oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides within a nucleic acid which encodes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, without hybridizing to any sequence of nucleotides within a nucleic acid which encodes the major surface antigen of a wildtype hepatitis B virus under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;

(ii) isolating the complex so formed; and

(iii) identifying the mRNA in the isolated complex so as to thereby determined whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

59. (Amended) The method of claim 57, wherein the nucleic acid sample in step (a)

comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and

(ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, wherein the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

61. (Amended) A method for use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein said method comprises:

(a) obtaining an appropriate sample from the subject; and

(b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 36 so as to

determine whether the subject has a predisposition for hepatocellular carcinoma.

62. (Amended) The method of claim 58, wherein the oligonucleotide or antibody is labeled with a detectable marker.

69. (Amended) A method comprising administering the composition of claim 47 as a medicament for treating hepatocellular carcinoma.

70. (Amended) A method comprising administering the composition of claim 67 as a medicament for treating hepatocellular carcinoma.

71. (Amended) A method comprising administering the composition of claim 47 as a medicament for preventing hepatocellular carcinoma.

72. (Amended) A method comprising administering the composition of claim 67 as a medicament for preventing hepatocellular carcinoma.

IN THE SPECIFICATION

Please insert the following Sequence Listing on a separate page immediately after page 36:

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Oon, Chong Jin
Lim, Gek Keow
Leong, Ai Lin
Zhao, Yi
Chen, Wei Ning

(ii) TITLE OF INVENTION: A VACCINE-INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Ladas & Parry
(B) STREET: 26 West 61 Street
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: USA
(F) ZIP: 10023

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:09/719,533
(B) FILING DATE:13-DEC-2000
(C) CLASSIFICATION:435

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/SG98/00045
(B) FILING DATE: 19-JUN-1998

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Mass, Clifford J.
(B) REGISTRATION NUMBER: 30,086
(C) REFERENCE/DOCKET NUMBER:U-013108-9

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 708-1890

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3215 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTCCACCACT TTCCACCAAA CTCTTCAAGA TCCCAGAGTC AGGGCCCTGT ACTTTCCTGC 60

TGGTGGCTCC AGTTCAGGAA CAGTGAGCCC TGCTCAGAAT ACTGTCTCTG CCATATCGTC 120

AATCTTATCG AAGACTGGGG ACCCTGTACC GAACATGGAG AACATCGCAT CAGGACTCCT 180

AGGACCCCTG CTCGTGTTAC AGGCGGGGTT TTTCTTGTTG ACAAAAATCC TCACAATACC 240

GCAGAGTCTA GACTCGTGGT GGACTTCTCT CAATTTTCTA GGGGGAACAC CCGTGTGTCT 300

TGGCCAAAAT TCGCAGTCCC AAATCTCCAG TCACTCACCA ACCTGTTGTC CTCCAATTTG 360

TCCTGGTTAT CGCTGGATGT GTCTGCGGCG TTTTATCATC TTCCTCTGCA TCCTGCTGCT 420

ATGCCTCATC TTCTTGTTGG TTCTTCTGGA CTATCAAGGT ATGTTGCCCG TTTGTCCTCT 480

AATTCCAGGA TCAACAACAA CCAGCACCGG ACCATGCAAA ACCTGCACAA CTCCTGCTCA 540

AGGAACCTCT ATGTTTCCCT CATGTTGCTG TACAAAACCT ACGGACAGAA ACTGCACCTG 600

TATTCCCATC CCATCATCTT GGGCTTTCGC AAAATACCTA TGGGAGTGGG CCTCAGTCCG 660

TTTCTCTTGG CTCAGTTTAC TAGTGCCATT TGTTCAGTGG TTCGTAGGGC TTTCCCCCAC 720

TGTCTGGCTT TCAGTTATAT GGATGATGTG GTTTTGGGGG CCAAGTCTGT ACAACATCTT 780

GAGTCCCTTT ATGCCGCTGT TACCAATTTT CTTTTGTCTT TGGGTATACA TTTAAACCCT 840

CACAAAACAA AAAGATGGGG ATATTCCCTT AACTTCATGG GATATGTCAT TGGGAGTTGG 900

GGCACATTGC CACAGGAACA TATTGTACAA AAAATCAAAA TGTGTTTTAG GAAACTTCCT 960

GTAAACAGGC CTATTGATTG GAAAGTATGT CAACGAATTG TGGGTCTTTT GGGGTTTGCC 1020

GCCCCTTTCA CGCAATGTGG ATATCCTGCT TTAATGCCTT TATATGCATG TATACAAGCA 1080

AAACAGGCTT TTACTTTCTC GCAAACTTAC AAGACCTTTC TAAGTAAACA GTATCTGAAC 1140

CTTTACCCCG TTGCTCGGCA ACGCCCTGGT CTGTGCCAAG TGTTTGCTGA CGCAACCCCC 1200

ACTGGTTGGG GCTTGGCCAT AGGCCATCAG CGCATGCGTG GAACCTTTGT GTCTCCTCTG 1260

CCGATCCATA CTGCGGAACT CCTAGCCGCT TGTTTTGCTC GCAGCAGGTC TGGGGCAAAA 1320

CTCATCGGGA CTGACAATTC TGTCGTGCTC TCCCGCAAGT ATACATCATT TCCATGGCTG 1380

CTAGGCTGTG CTGCCAACTG GATCCTGCGC GGGACGTCCT TTGTTTACGT CCCGTCGGCG 1440

CTGAATCCCG CGGACGACCC CTCCCGGGGC CGCTTGGGGC TCTACCGCCC GCTTCTCCGC 1500

CTGTTATACC GACCGACCAC GGGGCGCACC TCTCTTTACG CGGACTCCCC GTCTGTGCCT 1560

TCTCATCTGC CGGACCGTGT GCACTTCGCT TCACCTCTGC ACGTCGCATG GAGACCACCG 1620

TGAACGCCCA CGGGAACCTG CCCAAGGTCT TGCATAAGAG GACTCTTGGA CTTTCAGCAA 1680

TGTCAACGAC CGACCTTGAG GCATACTTCA AAGACTGTGT GTTTAATGAG TGGGAGGAGT 1740

TGGGGGAGGA GGTTAGGTTA AAGGTCTTTG TACTAGGAGG CTGTAGGCAT AAATTGGTGT 1800

GTTCACCATC ACCATGCAAC TTTTTCACCT CTGCCTAATC ATCTCATGTT CATGTCCTAC 1860

TGTTCAAGCC TCCAAGCTGT GCCTTGGGTG GCTTTGGGGC ATGGACATTG ACCCGTATAA 1920

AGAATTTGGA GCTTCTGTGG AGTTACTCTC TTTTTTGCCT TCTGACTTTT TTCCTTCTAT 1980

TCGAGATCTC CTCGACACCG CCTCTGCTCT GTATCGGGAG GCCTTAGAGT CTCCGGAACA 2040

TTGTTCACCT CACCATACGG CACTCAGGCA AGCTATTCTG AGTTGGGGTG AGTTAATGAA 2100

TCTAGCCACC TGGGTGGGAA GTAATTTGGA AGATCCAGCA TCCAGGGAAT TAGTAGTCAG 2160

CTATGTCAAC GTTAATATGG GCCTAAAAAT CAGACAACTA TTGTGGTTTC ACATTTCCTG 2220

TCTTACTTTT GGGAGAGAAA CTGTTCTTGA ATATTTGGTG TCTTTTGGAG TGTGGATTCG 2280

CACTCCTCCT GCATATAGAC CACCAAATGC CCCTATCTTA TCAACACTTC CGGAAACTAC 2340

TGTTGTTAGA CGAAGAGGCA GGTCCCCTAG AAGAAGAACT CCCTCGCCTC GCAGACGAAG 2400

GTCTCAATCG CCGCGTCGCA GAAGATCTCA ATCTCGGGAA TCTCAATGTT AGTATTCCTT 2460

GGACACATAA GGTGGGAAAC TTTACGGGGC TTTATTCTTC TACGGTACCT TGCTTTAATC 2520

CTAAATGGCA AACTCCTTCT TTTCCGGACA TTCATTTGCA GGAGGACATT CTTGATAGAT 2580

GTAAGCAATT TGTGGGGCCC CTTACAGTAA ATGAAAACAG GAGACTAAAA TTAATTATGC 2640

CTGCTAGGTT TTATCCAAAT GTTACTAAAT ATTTGCCCTT AGATAAAGGG ATCAAACCAT 2700

ATTATCCAGA GTATGTAGTT AATCATTACT TCCAGACGCG ACATTATTTA CACACTCTTT 2760

GGAAGGCGGG GATCTTATAT AAAAGAGAGT CCACACGTAG CGCCTCATTT TGCGGGTCAC 2820

CATATTCTTG GGAACAAGAT CTACAGCATG GGAGGTTGGT CTTCCAAACC TCGAAAAGGC 2880

ATGGGGACAA ATCTTTCTGT CCCCAATCCC CTGGGATTCT TCCCCGATCA TCAGTTGGAC 2940

CCTGCATTCA AAGCCAACTC AGAAAATCCA GATTGGGACC TCAACCCGCA CAAGGACAAC 3000

TGGCCGGACG CCAACAAGGT GGGAGTGGGA GCATTCGGGC CAGGGTTCAC CCCTCCTCAT 3060

GGGGGACTGT TGGGGTGGAG CCCTCAGGCT CAGGGCCTAC TCACAACTGT GCCAGCAGCT 3120

CCTCCTCCTG CCTCCACCAA TCGGCAGTCA GGAAGGCAGC CTACTCCCTT ATCTCCACCT 3180

CTAAGGGACA CTCATCCTCA GGCCATGCAG TGGAA 3215

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 843 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Pro Leu Ser Tyr Gln His Phe Arg Lys Leu Leu Leu Leu Asp Glu
1               5                  10                  15

Glu Ala Gly Pro Leu Glu Glu Glu Leu Pro Arg Leu Ala Asp Glu Gly
            20                  25                  30

Leu Asn Arg Arg Val Ala Glu Asp Leu Asn Leu Gly Asn Leu Asn Val
        35                  40                  45

Ser Ile Pro Trp Thr His Lys Val Gly Asn Phe Thr Gly Leu Tyr Ser
    50                  55                  60

Ser Thr Val Pro Cys Phe Asn Pro Lys Trp Gln Thr Pro Ser Phe Pro
65                  70                  75                  80

Asp Ile His Leu Gln Glu Asp Ile Leu Asp Arg Cys Lys Gln Phe Val
                85                  90                  95

Glu Pro Leu Thr Val Asn Glu Asn Arg Arg Leu Lys Leu Ile Met Pro
            100                 105                 110

Ala Arg Phe Tyr Pro Asn Val Thr Lys Tyr Leu Pro Leu Asp Lys Gly
        115                 120                 125

Ile Lys Pro Tyr Tyr Pro Glu Tyr Val Val Asn His Tyr Phe Gln Thr
    130                 135                 140

Arg His Tyr Leu His Thr Leu Trp Lys Ala Gly Ile Leu Tyr Lys Arg
145                 150                 155                 160

Glu Ser Thr Arg Ser Ala Ser Phe Cys Gly Ser Pro Tyr Ser Trp Glu
                165                 170                 175

Gln Asp Leu Gln His Gly Arg Leu Val Phe Gln Thr Ser Lys Arg His
            180                 185                 190

Gly Asp Lys Ser Phe Cys Pro Glu Ser Pro Gly Ile Leu Pro Arg Ser
        195                 200                 205

Ser Val Gly Pro Cys Ile Gln Ser Gln Leu Arg Lys Ser Arg Leu Gly
    210                 215                 220

Pro Gln Pro Ala Gln Gly Gln Leu Ala Gly Arg Gln Gln Gly Gly Ser
225                 230                 235                 240
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Gly Ser Ile Arg Ala Arg Val His Pro Ser Ser Trp Gly Thr Val Gly
245 250 255

Val Glu Pro Ser Gly Ser Gly Pro Thr His Asn Cys Ala Ser Ser Ser
260 265 270

Ser Ser Cys Leu His Gln Ser Ala Val Arg Lys Ala Ala Tyr Ser Leu
275 280 285

Ile Ser Thr Ser Lys Gly His Ser Ser Ser Gly His Ala Val Glu Leu
290 295 300

His His Phe Pro Pro Asn Ser Ser Arg Ser Gln Ser Gln Gly Pro Val
305 310 315 320

Leu Ser Cys Trp Trp Leu Gln Phe Arg Asn Ser Glu Pro Cys Ser Glu
325 330 335

Tyr Cys Leu Cys His Ile Val Asn Leu Ile Glu Asp Trp Gly Pro Cys
340 345 350

Thr Glu His Gly Glu His Arg Ile Arg Thr Pro Arg Thr Pro Ala Arg
355 360 365

Val Thr Gly Gly Val Phe Leu Val Asp Lys Asn Pro His Asn Thr Ala
370 375 380

Glu Ser Arg Leu Val Val Asp Phe Ser Gln Phe Ser Arg Gly Asn Thr
385 390 395 400

Arg Val Ser Trp Pro Lys Phe Ala Val Pro Asn Leu Gln Ser Leu Thr
405 410 415

Asn Leu Leu Ser Ser Asn Leu Ser Trp Leu Ser Leu Asp Val Ser Ala
420 425 430

Ala Phe Tyr His Leu Pro Leu His Pro Ala Ala Met Pro His Leu Leu
435 440 445

Val Gly Ser Ser Gly Leu Ser Arg Tyr Val Ala Arg Leu Ser Ser Asn
450 455 460

Ser Arg Ile Asn Asn Asn Glu His Arg Thr Met Glu Asn Leu His Asn
465 470 475 480

Ser Cys Ser Arg Asn Leu Tyr Val Ser Leu Met Leu Leu Tyr Lys Thr
485 490 495

Tyr Gly Gln Lys Leu His Leu Tyr Ser His Pro Ile Ile Leu Gly Phe
500 505 510

Arg Lys Ile Pro Met Gly Val Gly Leu Ser Pro Phe Leu Leu Ala Gln
515 520 525

Phe Thr Ser Ala Ile Cys Ser Val Val Arg Arg Ala Phe Pro His Cys

```
    530                 535                 540

Leu Ala Phe Ser Tyr Met Asp Asp Val Val Leu Gly Ala Lys Ser Val
545                 550                 555                 560

Gln His Leu Glu Ser Leu Tyr Ala Ala Val Thr Asn Phe Leu Leu Ser
                565                 570                 575

Leu Gly Ile His Leu Asn Pro His Lys Thr Lys Arg Trp Gly Tyr Ser
            580                 585                 590

Leu Asn Phe Met Gly Tyr Val Ile Gly Ser Trp Gly Thr Leu Pro Gln
        595                 600                 605

Glu His Ile Val Gln Lys Ile Lys Met Cys Phe Arg Lys Leu Pro Val
    610                 615                 620

Asn Arg Pro Ile Asp Trp Lys Val Cys Gln Arg Ile Val Gly Leu Leu
625                 630                 635                 640

Gly Phe Ala Ala Pro Phe Thr Gln Cys Gly Tyr Pro Ala Leu Met Pro
                645                 650                 655

Leu Tyr Ala Cys Ile Gln Ala Lys Gln Ala Phe Thr Phe Ser Gln Thr
            660                 665                 670

Tyr Lys Thr Phe Leu Ser Lys Gln Tyr Leu Asn Leu Tyr Pro Val Ala
        675                 680                 685

Arg Gln Arg Pro Gly Leu Cys Glu Val Phe Ala Asp Ala Thr Pro Thr
    690                 695                 700

Gly Trp Gly Leu Ala Ile Gly His Gln Arg Met Arg Gly Thr Phe Val
705                 710                 715                 720

Ser Pro Leu Pro Ile His Thr Ala Glu Leu Leu Ala Ala Cys Phe Ala
                725                 730                 735

Arg Ser Arg Ser Gly Ala Lys Leu Ile Gly Thr Asp Asn Ser Val Val
            740                 745                 750

Leu Ser Arg Lys Tyr Thr Ser Phe Pro Trp Leu Leu Gly Cys Ala Ala
        755                 760                 765

Asn Trp Ile Leu Arg Gly Thr Ser Phe Val Tyr Val Pro Ser Ala Leu
    770                 775                 780

Asn Pro Ala Asp Asp Pro Ser Arg Gly Arg Leu Gly Leu Tyr Arg Pro
785                 790                 795                 800

Leu Leu Arg Leu Leu Tyr Arg Pro Thr Thr Gly Arg Thr Ser Leu Tyr
                805                 810                 815

Ala Asp Ser Pro Ser Val Pro Ser His Leu Pro Asp Arg Val His Phe
```

820 825 830

Ala Ser Pro Leu His Val Ala Trp Arg Pro Pro
835 840

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 400 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Gly Gly Trp Ser Ser Lys Pro Arg Lys Gly Met Gly Thr Asn Leu
1 5 10 15

Ser Val Pro Asn Pro Leu Gly Phe Phe Pro Asp His Gln Leu Asp Pro
20 25 30

Ala Phe Lys Ala Asn Ser Glu Asn Pro Asp Trp Asp Leu Asn Pro His
35 40 45

Lys Asp Asn Trp Pro Asp Ala Asn Lys Val Gly Val Gly Ala Phe Gly
50 55 60

Pro Gly Phe Thr Pro Pro His Gly Gly Leu Leu Gly Trp Ser Pro Gln
65 70 75 80

Ala Gln Gly Leu Leu Thr Thr Val Pro Ala Ala Pro Pro Pro Ala Ser
85 90 95

Thr Asn Arg Gln Ser Gly Arg Gln Pro Thr Pro Leu Ser Pro Pro Leu
100 105 110

Arg Asp Thr His Pro Gln Ala Met Gln Trp Asn Ser Thr Thr Phe His
115 120 125

Gln Thr Leu Gln Asp Pro Arg Val Arg Ala Leu Tyr Phe Pro Ala Gly
130 135 140

Gly Ser Ser Ser Gly Thr Val Ser Pro Ala Gln Asn Thr Val Ser Ala
145 150 155 160

Ile Ser Ser Ile Leu Ser Lys Thr Gly Asp Pro Val Pro Asn Met Glu
165 170 175

Asn Ile Ala Ser Gly Leu Leu Gly Pro Leu Leu Val Leu Gln Ala Gly
180 185 190

```
Phe Phe Leu Leu Thr Lys Ile Leu Thr Ile Pro Gln Ser Leu Asp Ser
        195                 200                 205

Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Pro Thr Val Cys Leu Gly
    210                 215                 220

Gln Asn Ser Gln Ser Gln Ile Ser Ser His Ser Pro Thr Cys Cys Pro
225                 230                 235                 240

Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile Ile
                245                 250                 255

Phe Leu Cys Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu
            260                 265                 270

Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser Thr
        275                 280                 285

Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln Gly
    290                 295                 300

Thr Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Thr Asp Arg Asn
305                 310                 315                 320

Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys Tyr Leu
                325                 330                 335

Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val Pro
            340                 345                 350

Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser Val
        355                 360                 365

Ile Trp Met Met Trp Phe Trp Gly Pro Ser Leu Tyr Asn Ile Leu Ser
    370                 375                 380

Pro Phe Met Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr Ile
385                 390                 395                 400
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 212 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Gln Leu Phe His Leu Cys Leu Ile Ile Ser Cys Ser Cys Pro Thr
1               5                   10                  15
```

```
Val Gln Ala Ser Lys Leu Cys Leu Gly Trp Leu Trp Gly Met Asp Ile
            20                  25                  30

Asp Pro Tyr Lys Glu Phe Gly Ala Ser Val Glu Leu Leu Ser Phe Leu
        35                  40                  45

Pro Ser Asp Phe Phe Pro Ser Ile Arg Asp Leu Leu Asp Thr Ala Ser
    50                  55                  60

Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys Ser Pro His
65                  70                  75                  80

His Thr Ala Leu Arg Gln Ala Ile Leu Ser Trp Gly Glu Leu Met Asn
                85                  90                  95

Leu Ala Thr Trp Val Gly Ser Asn Leu Glu Asp Pro Ala Ser Arg Glu
            100                 105                 110

Leu Val Val Ser Tyr Val Asn Val Asn Met Gly Leu Lys Ile Arg Gln
        115                 120                 125

Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg Glu Thr Val
    130                 135                 140

Leu Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr Pro Pro Ala
145                 150                 155                 160

Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro Glu Thr Thr
                165                 170                 175

Val Val Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr Pro Ser Pro
            180                 185                 190

Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser Gln Ser Arg
        195                 200                 205

Glu Ser Gln Cys
    210
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 154 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Ala Ala Arg Leu Cys Cys Gln Leu Asp Pro Ala Arg Asp Val Leu
1               5                   10                  15

Cys Leu Arg Pro Val Gly Ala Glu Ser Arg Gly Arg Pro Leu Pro Gly
            20                  25                  30
```

Pro Leu Gly Ala Leu Pro Pro Ala Ser Pro Pro Val Ile Pro Thr Asp
35 40 45

His Gly Ala His Leu Ser Leu Arg Gly Leu Pro Val Cys Ala Phe Ser
50 55 60

Ser Ala Gly Pro Cys Ala Leu Arg Phe Thr Ser Ala Arg Arg Met Glu
65 70 75 80

Thr Thr Val Asn Ala His Gly Asn Leu Pro Lys Val Leu His Lys Arg
85 90 95

Thr Leu Gly Leu Ser Ala Met Ser Thr Thr Asp Leu Glu Ala Tyr Phe
100 105 110

Lys Asp Cys Val Phe Asn Glu Trp Glu Glu Leu Gly Glu Glu Val Arg
115 120 125

Leu Lys Val Phe Val Leu Gly Gly Cys Arg His Lys Leu Val Cys Ser
130 135 140

Pro Ser Pro Cys Asn Phe Phe Thr Ser Ala
145 150

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATAAGCTTATG CCCCTATCTT ATCAACACTT CCGGA 35

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAGTCTAGAC TCTGCGGTAT TGTGA 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAGTCTAGAC TCGTGGTGGA CTTCT 25

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGAGAATTCT CACGGTGGTC TCCATGCGAC GT 32

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TTTGTTTACG TCCCGT 16

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTAAGCTTAG TTTCCGGAAG TGTTGAT

REMARKS

The above amendatory action to the claims is taken solely for the purpose of avoiding claim fees that would otherwise accrue due to the presence of multiple dependent claims, with the exception that the Accession Nos. in claims 1, 21, 22 and 24 have been amended based on the disclosure in the Abstract of International Publication WO 99/66047 (PCT/SG98/00045) to correct an inadvertant clerical error.

The amendatory action to the specification is taken to comply with the applicable sequence listing requirements. A computer readable copy of the Sequence Listing and the requisite statement are submitted herewith.

Respectfully submitted,

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c/o LADAS & PARRY
26 WEST 61ST STREET
NEW YORK, NEW YORK 10023
REG. NO.: 30,086 (212) 708-1890

MARKED-UP COPY

1. (Amended) An isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) and deposited under Accession Nos. P971215014, P971215025 and P971215036 with the European Collection of Cell Culture on 15th December 1997.

14. (Amended) The vector of claim 12 or 13, wherein the vector comprises viral DNA.

21. (Amended) A purified polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P971215014, P971215025 and P971215036 with the European Collection of Cell Culture on 15th December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.

22. (Amended) A purified polypeptide obtained from a method which comprises:

(a) introducing a vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P971215014, P971215025 and P971215036 with the European Collection of Cell Culture on 15th December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine and operatively linked to a promoter of RNA transcription into a suitable host cell;

(b) culturing the resulting host cell so as to produce the polypeptide;

(c) recovering the polypeptide produced in step (b); and

(d) purifying the polypeptide so recovered.

24. (Amended) A purified peptide obtained from a method which comprises:

(a) introducing a vector comprising an isolated nuclei acid encoding a peptide which comprises at least a portion of a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P9712150~~1~~4, P971215025 and P971215036 with the European Collection of Cell Culture on 15th December 1997 wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1 into a suitable host cell;

(b) culturing the resulting host cell so as to produce the polypeptide;

(c) recovering the polypeptide produced in step (b); and

(d) purifying the polypeptide so recovered.

33. (Amended) The antibodies obtained in claim 27 or 31.

37. U(Amended)A method for use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such determinationmethod comprises

(a) obtaining an appropriate nucleic acid sample from the subject; and

(b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine.

38. The use of claim 37(Amended) A method for use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a

major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such method comprises

(a) obtaining an appropriate nucleic acid sample from the subject; and

(b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;

(ii) isolating the complex so formed; and

(iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

39. (Amended) The use method of claim 37, wherein the nucleic acid sample in step

(a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the

amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and

(ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by thean isolated nucleic acid of claim 9encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, wherein the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

40. (Amended) The usemethod of claim 37, wherein the determining of step (b) comprises:

(i) amplifying the nucleic acid present in the sample of step (a); and

(ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

41. U(Amended) A method of use of antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such determinationmethod comprises:

(a) obtaining an appropriate sample from the subject; and

(b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 or 36 so as to determine whether a subject is infected.

42. (Amended) The usemethod of claim 37, 38 or 41, wherein the isolated nucleic acid, oligonucleotide, or antibody is labeled with a detectable marker.

43. (Amended) The use themethod of claim 42, wherein the detectable marker is a radioactive isotope, a fluorophor, or an enzyme.

44. (Amended) The usemethod of claim 37, wherein the sample comprises blood, tissue, or sera.

51. Use of(Amended) A method comprising administering the composition of claim 47 or 48 for treating a subject infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).

52. Use of(Amended) A method comprising administering the composition of claim 49 for treating a subject infected with a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).

53. Use of(Amended) A method comprising administering the composition of claim 47 or 48 for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject.

54. Use of(Amended) A method comprising administering the composition of claim 50 for preventing infection with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject.

57. U(Amended) A method for use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determinationsaid method comprises:

(a) obtaining an appropriate nucleic acid sample from the subject; and

(b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.

58. (Amended) The method of claim 57, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) contacting the mRNA with thean oligonucleotide of claim 25 under conditionsat least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides within a nucleic acid which encodes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major

surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, without hybridizing to any sequence of nucleotides within a nucleic acid which encodes the major surface antigen of a wildtype hepatitis B virus under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;

(ii) isolating the complex so formed; and

(iii) identifying the mRNA in the isolated complex so as to thereby determined whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

59. (Amended) The method of claim 57, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and

(ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by thean isolated nucleic acid of claim 9encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, wherein the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

61. U(Amended) A method for use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determinationsaid method comprises:

(a) obtaining an appropriate sample from the subject; and

(b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 36 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.

62. (Amended) The method of claim 58, 59 or 60, wherein the oligonucleotide or antibody is labeled with a detectable marker.

69. Use of(Amended) A method comprising administering the composition of claim 47 or 48 as a medicament for treating hepatocellular carcinoma.

70. Use of(Amended) A method comprising administering the composition of claim 67 as a medicament for treating hepatocellular carcinoma.

71. Use of(Amended) A method comprising administering the composition of claim 47 or 48 as a medicament for preventing hepatocellular carcinoma.

72. Use of(Amended) A method comprising administering the composition of claim 67 as a medicament for preventing hepatocellular carcinoma.

#3

09/719533

Practitioner's Docket No. U 013108-9

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Chong Jin OON, et al.
Serial No.:09/719,533 Group No.: --
Filed: December 13, 2000 Examiner: --
For: A VACCINE INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF

Box Sequence
Assistant Commissioner for Patents
Washington, D.C. 20231

SUBMISSION OF "SEQUENCE LISTING," COMPUTER READABLE COPY, AND/OR AMENDMENT PERTAINING THERETO FOR BIOTECHNOLOGY INVENTION CONTAINING NUCLEOTIDE AND/OR AMINO ACID SEQUENCE

CERTIFICATION UNDER 37 C.F.R. 1.8(a) and 1.10*
*(When using Express Mail, the Express Mail label number is **mandatory**; Express Mail certification is optional.)*

I hereby certify that, on the date shown below, this correspondence is being:

MAILING

☒ deposited with the United States Postal Service in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

37 C.F.R. 1.8(a)

☐ with sufficient postage as first class mail.

37 C.F.R. 1.10*

☒ as "Express Mail Post Office to Address" Mailing Label No. EL728213866US **(mandatory)**

TRANSMISSION

☐ transmitted by facsimile to the Patent and Trademark Office.

Date: July 10, 2001

Jennifer Rashkin
Signature

JENNIFER RASHKIN
(type or print name of person certifying)

***WARNING:** *Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

(check and complete this item, if applicable)

1. [X] This replies to the Office Letter DATED April 30, 2001 .

NOTE: If these papers are filed before the office letter issues, adequate identification of the original papers should be made, e.g., in addition to the name of the inventor and title of invention, the filing date based on the "Express Mail" procedure, the serial number from the return post card or the attorney's docket number added.

[X] A copy of the Office Letter is enclosed.

IDENTIFICATION OF PERSON MAKING STATEMENT

2. I, Clifford J. Mass
(type or print name of person signing below)

state the following:

ITEMS BEING SUBMITTED

3. Submitted herewith is/are

(check each item as applicable)

A. ☒ "Sequence Listing(s)" for the nucleotide and/or amino acid sequence(s) in this application. Each "Sequence Listing" is assigned a separate identifier as required in 37 C.F.R. § 1.821(c) and 37 C.F.R. §§ 1.822 and 1.823.

B. [ ] An amendment to the description and/or claims, wherein reference is made to the sequence by use of the assigned identifier, as required in 37 C.F.R. § 1.821(d).

C. ☒ A copy of each "Sequence Listing" submitted for this application in computer readable form, in accordance with the requirements of 37 C.F.R. §§ 1.821(e) and 1.824.

D. [ ] Please transfer to this application, in accordance with 37 C.F.R. § 1.821(e), the computer readable copy(ies) from applicant's other application identified as follows:

In re application of:
Serial No.: Group No.:
Filed: Examiner:
For:

The Computer readable form(s) of applicant's other application corresponds to the "Sequence Identifier(s)" of the application as follows:

Computer Readable Form	"Sequence Identifier"
(other applications)	(this application)

NOTE: "If the computer readable form of a new application is to be identical with the computer readable form of another application of the applicant on file in the Office, reference maybe made to the other application and computer readable form in lieu of filing a duplicate computer readable form in the new application. The new application shall be accompanied by a letter making such reference to the other application and computer readable form, both of which shall be completely identified." 37 C.F.R. 1.821(e).

E. ☒ A statement that the content of each "Sequence Listing" submitted and each computer readable copy are the same, as required in 37 C.F.R. § 1.821(f).

[ ] Because the statement is not made by a person registered to practice before the Office, the Statement is verified as required in 37 C.F.R. § 1.821(b).

F. ☒ Because this submission is made in fulfilling the requirement under 37 C.F.R. § 1.821(g), a statement that the submission includes no new matter.

[ ] Because the statement is not made by a person registered to practice before the Office, the statement is verified, as required in 37 C.F.R. § 1.821(g).

**STATEMENT THAT "SEQUENCE LISTING"
AND COMPUTER READABLE COPY ARE THE SAME
AND/OR THAT PAPERS SUBMITTED INCLUDES NO NEW MATTER**

4. I hereby state:

(complete applicable item A and/or B)

A. ☒ Each computer readable form submitted in this application, including those forms requested to be transferred from applicant's other application, is the same as the "Sequence Listing" to which it is indicated to relate.

B. ☒ All papers accompanying this submission, or for which a request for transfer from applicants' other application, introduce no new matter.

STATUS

5. Applicant is

[ ] a small entity. A statement:

[ ] is attached.

[ ] was already filed.

☒ other than a small entity.

EXTENSION OF TERM

6.

NOTE: "Extension of Time in Patent Cases (Supplement Amendments) If a timely and complete response has been filed after a Non-Final Office Action, an extension of time is not required to permit filing and/or entry of an additional amendment after expiration of the shortened statutory period.

If a timely response has been filed after a Final Office Action, an extension of time is required to permit filing and/or entry of a Notice of Appeal or filing and/or entry of an additional amendment after expiration of the shortened statutory period unless the timely-filed response placed the application in condition for allowance. Of course, if a Notice of Appeal has been filed within the shortened statutory period, the period has ceased to run." Notice of Dec. 10, 1985 (1061 O.G. 34-35).

NOTE: See 37 C.F.R. 1.645 for extensions of time in interference proceedings and 37 C.F.R. 1.550(c) for extensions of time in reexamination proceedings.

7. The proceedings herein are for a patent application and the provisions of 37 C.F.R. 1.136 apply.

(complete (a) or (b) as applicable)

(a) [X] Applicant petitions for an extension of time under 37 C.F.R. 1.136 (fees: 37 C.F.R. 1.17(a)(1)-(4)) for the total number of months checked below:

	Extension (months)	Fee for other than small entity	Fee for small entity
[X]	one month	$110.00	$ 55.00
[ ]	two months	$390.00	$ 195.00
[ ]	three months	$890.00	$ 445.00
[ ]	four months	$1,390.00	$ 695.00

Fee $110.00 is being paid concurrently in the Completion submitted herewith

If an additional extension of time is required, please consider this a petition therefor.

(check and complete the next item, if applicable)

[ ] An extension for _____ months has already been secured, and the fee paid therefor of $ _____ is deducted from the total fee due for the total months of extension now requested.

Extension fee due with this request $_____

OR

(b) [ ] Applicant believes that no extension of term is required. However, this conditional petition is being made to provide for the possibility that applicant has inadvertently overlooked the need for a petition and fee for extension of time.

FEE PAYMENT

8. [X] Attached is a check in the sum of is being paid concurrently in the Completion submitted herewith.

[ ] Charge Account No. _____ the sum of $ _____.
A duplicate of this transmittal is attached.

FEE DEFICIENCY

9.

NOTE: If there is a fee deficiency and there is no authorization to charge an account, additional fees are necessary to cover the additional time consumed in making up the original deficiency. If the maximum, six-month period has expired before the deficiency is noted and corrected, the application is held abandoned. In those instances where authorization to charge is included, processing delays are encountered in returning the papers to the PTO finance Branch in order to apply these charges prior to action on the cases. Authorization to charge the deposit account for any fee deficiency should be checked. See the Notice of April 7, 1986, 1065 O.G. 31-33.

10. [X] If any additional extension and/or fee is required, charge Account No. 12-0425.

SIGNATURE(s)

Clifford J. Mass
(type or print name of person signing statement)

Signature

July , 2001
Date

Clifford J. Mass
P.O. Address of Signatory Registration No. 30,086
c/o LADAS & PARRY
26 West 61st Street
New York, NY 10023
(If applicable) (212) 708-1890

Tel. No.: (212)

Reg. No.

[ ] Inventor
[ ] Assignee of complete interest
[ ] Person authorized to sign on behalf of assignee
[ ] Practitioner of record
☒ Filed under Rule 34(a)
[ ] Registration No. _____
[ ] Other _____
(specify identity of person signing)

(complete the following, if applicable)

_____
(type name of assignee)

_____
Address of assignee

_____

_____
Title of person authorized to sign on behalf of assignee

A "STATEMENT UNDER 37 C.F.R. 3.73(b)" is attached.

Assignment recorded in PTO on _____
Reel _____ Frame _____

Reg. No.

Tel. No.: ( )

Customer No.:

_____
SIGNATURE OF PRACTITIONER

Clifford J. Mass Reg No 30,086
(type or print name of practitioner)

_____
P.O. Address

_____
c/o Ladas & Parry
26 West 61st Street
New York, N.Y. 10023

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Oon, Chong Jin
Lim, Gek Keow
Leong, Ai Lin
Zhao, Yi
Chen, Wei Ning

(ii) TITLE OF INVENTION: A VACCINE-INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Ladas & Parry
(B) STREET: 26 West 61 Street
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: USA
(F) ZIP: 10023

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:09/719,533
(B) FILING DATE:13-DEC-2000
(C) CLASSIFICATION:435

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/SG98/00045
(B) FILING DATE: 19-JUN-1998

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Mass, Clifford J.
(B) REGISTRATION NUMBER: 30,086
(C) REFERENCE/DOCKET NUMBER:U-013108-9

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 708-1890

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3215 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTCCACCACT TTCCACCAAA CTCTTCAAGA TCCCAGAGTC AGGGCCCTGT ACTTTCCTGC 60

TGGTGGCTCC AGTTCAGGAA CAGTGAGCCC TGCTCAGAAT ACTGTCTCTG CCATATCGTC 120

AATCTTATCG AAGACTGGGG ACCCTGTACC GAACATGGAG AACATCGCAT CAGGACTCCT 180

AGGACCCCTG CTCGTGTTAC AGGCGGGGTT TTTCTTGTTG ACAAAAATCC TCACAATACC 240

GCAGAGTCTA GACTCGTGGT GGACTTCTCT CAATTTTCTA GGGGGAACAC CCGTGTGTCT 300

TGGCCAAAAT TCGCAGTCCC AAATCTCCAG TCACTCACCA ACCTGTTGTC CTCCAATTTG 360

TCCTGGTTAT CGCTGGATGT GTCTGCGGCG TTTTATCATC TTCCTCTGCA TCCTGCTGCT 420

ATGCCTCATC TTCTTGTTGG TTCTTCTGGA CTATCAAGGT ATGTTGCCCG TTTGTCCTCT 480

AATTCCAGGA TCAACAACAA CCAGCACCGG ACCATGCAAA ACCTGCACAA CTCCTGCTCA 540

AGGAACCTCT ATGTTTCCCT CATGTTGCTG TACAAAACCT ACGGACAGAA ACTGCACCTG 600

TATTCCCATC CCATCATCTT GGGCTTTCGC AAAATACCTA TGGGAGTGGG CCTCAGTCCG 660

TTTCTCTTGG CTCAGTTTAC TAGTGCCATT TGTTCAGTGG TTCGTAGGGC TTTCCCCCAC 720

TGTCTGGCTT TCAGTTATAT GGATGATGTG GTTTTGGGGG CCAAGTCTGT ACAACATCTT 780

GAGTCCCTTT ATGCCGCTGT TACCAATTTT CTTTTGTCTT TGGGTATACA TTTAAACCCT 840

CACAAAACAA AAAGATGGGG ATATTCCCTT AACTTCATGG GATATGTCAT TGGGAGTTGG 900

GGCACATTGC CACAGGAACA TATTGTACAA AAAATCAAAA TGTGTTTTAG GAAACTTCCT 960

GTAAACAGGC CTATTGATTG GAAAGTATGT CAACGAATTG TGGGTCTTTT GGGGTTTGCC 1020

GCCCCTTTCA CGCAATGTGG ATATCCTGCT TTAATGCCTT TATATGCATG TATACAAGCA 1080

AAACAGGCTT TTACTTTCTC GCAAACTTAC AAGACCTTTC TAAGTAAACA GTATCTGAAC 1140

CTTTACCCCG TTGCTCGGCA ACGCCCTGGT CTGTGCCAAG TGTTTGCTGA CGCAACCCCC 1200

ACTGGTTGGG GCTTGGCCAT AGGCCATCAG CGCATGCGTG GAACCTTTGT GTCTCCTCTG 1260

CCGATCCATA CTGCGGAACT CCTAGCCGCT TGTTTTGCTC GCAGCAGGTC TGGGGCAAAA 1320

CTCATCGGGA CTGACAATTC TGTCGTGCTC TCCCGCAAGT ATACATCATT TCCATGGCTG 1380

CTAGGCTGTG CTGCCAACTG GATCCTGCGC GGGACGTCCT TTGTTTACGT CCCGTCGGCG 1440

CTGAATCCCG CGGACGACCC CTCCCGGGGC CGCTTGGGGC TCTACCGCCC GCTTCTCCGC 1500

CTGTTATACC GACCGACCAC GGGGCGCACC TCTCTTTACG CGGACTCCCC GTCTGTGCCT 1560

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TGAACGCCCA CGGGAACCTG CCCAAGGTCT TGCATAAGAG GACTCTTGGA CTTTCAGCAA 1680

TGTCAACGAC CGACCTTGAG GCATACTTCA AAGACTGTGT GTTTAATGAG TGGGAGGAGT 1740

TGGGGGAGGA GGTTAGGTTA AAGGTCTTTG TACTAGGAGG CTGTAGGCAT AAATTGGTGT 1800

GTTCACCATC ACCATGCAAC TTTTTCACCT CTGCCTAATC ATCTCATGTT CATGTCCTAC 1860

TGTTCAAGCC TCCAAGCTGT GCCTTGGGTG GCTTTGGGGC ATGGACATTG ACCCGTATAA 1920

AGAATTTGGA GCTTCTGTGG AGTTACTCTC TTTTTTGCCT TCTGACTTTT TTCCTTCTAT 1980

TCGAGATCTC CTCGACACCG CCTCTGCTCT GTATCGGGAG GCCTTAGAGT CTCCGGAACA 2040

TTGTTCACCT CACCATACGG CACTCAGGCA AGCTATTCTG AGTTGGGGTG AGTTAATGAA 2100

TCTAGCCACC TGGGTGGGAA GTAATTTGGA AGATCCAGCA TCCAGGGAAT TAGTAGTCAG 2160

CTATGTCAAC GTTAATATGG GCCTAAAAAT CAGACAACTA TTGTGGTTTC ACATTTCCTG 2220

TCTTACTTTT GGGAGAGAAA CTGTTCTTGA ATATTTGGTG TCTTTTGGAG TGTGGATTCG 2280

CACTCCTCCT GCATATAGAC CACCAAATGC CCCTATCTTA TCAACACTTC CGGAAACTAC 2340

TGTTGTTAGA CGAAGAGGCA GGTCCCCTAG AAGAAGAACT CCCTCGCCTC GCAGACGAAG 2400

GTCTCAATCG CCGCGTCGCA GAAGATCTCA ATCTCGGGAA TCTCAATGTT AGTATTCCTT 2460

GGACACATAA GGTGGGAAAC TTTACGGGGC TTTATTCTTC TACGGTACCT TGCTTTAATC 2520

CTAAATGGCA AACTCCTTCT TTTCCGGACA TTCATTTGCA GGAGGACATT CTTGATAGAT 2580

GTAAGCAATT TGTGGGGCCC CTTACAGTAA ATGAAAACAG GAGACTAAAA TTAATTATGC 2640

CTGCTAGGTT TTATCCAAAT GTTACTAAAT ATTTGCCCTT AGATAAAGGG ATCAAACCAT 2700

ATTATCCAGA GTATGTAGTT AATCATTACT TCCAGACGCG ACATTATTTA CACACTCTTT 2760

GGAAGGCGGG GATCTTATAT AAAAGAGAGT CCACACGTAG CGCCTCATTT TGCGGGTCAC 2820

CATATTCTTG GGAACAAGAT CTACAGCATG GGAGGTTGGT CTTCCAAACC TCGAAAAGGC 2880

ATGGGGACAA ATCTTTCTGT CCCCAATCCC CTGGGATTCT TCCCCGATCA TCAGTTGGAC 2940

CCTGCATTCA AAGCCAACTC AGAAAATCCA GATTGGGACC TCAACCCGCA CAAGGACAAC 3000

TGGCCGGACG CCAACAAGGT GGGAGTGGGA GCATTCGGGC CAGGGTTCAC CCCTCCTCAT 3060

GGGGGACTGT TGGGGTGGAG CCCTCAGGCT CAGGGCCTAC TCACAACTGT GCCAGCAGCT 3120

CCTCCTCCTG CCTCCACCAA TCGGCAGTCA GGAAGGCAGC CTACTCCCTT ATCTCCACCT 3180

CTAAGGGACA CTCATCCTCA GGCCATGCAG TGGAA 3215

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 843 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Pro Leu Ser Tyr Gln His Phe Arg Lys Leu Leu Leu Leu Asp Glu
1               5                  10                  15

Glu Ala Gly Pro Leu Glu Glu Glu Leu Pro Arg Leu Ala Asp Glu Gly
            20                  25                  30

Leu Asn Arg Arg Val Ala Glu Asp Leu Asn Leu Gly Asn Leu Asn Val
        35                  40                  45

Ser Ile Pro Trp Thr His Lys Val Gly Asn Phe Thr Gly Leu Tyr Ser
    50                  55                  60

Ser Thr Val Pro Cys Phe Asn Pro Lys Trp Gln Thr Pro Ser Phe Pro
65                  70                  75                  80

Asp Ile His Leu Gln Glu Asp Ile Leu Asp Arg Cys Lys Gln Phe Val
                85                  90                  95

Glu Pro Leu Thr Val Asn Glu Asn Arg Arg Leu Lys Leu Ile Met Pro
            100                 105                 110

Ala Arg Phe Tyr Pro Asn Val Thr Lys Tyr Leu Pro Leu Asp Lys Gly
        115                 120                 125

Ile Lys Pro Tyr Tyr Pro Glu Tyr Val Val Asn His Tyr Phe Gln Thr
    130                 135                 140

Arg His Tyr Leu His Thr Leu Trp Lys Ala Gly Ile Leu Tyr Lys Arg
145                 150                 155                 160

Glu Ser Thr Arg Ser Ala Ser Phe Cys Gly Ser Pro Tyr Ser Trp Glu
                165                 170                 175

Gln Asp Leu Gln His Gly Arg Leu Val Phe Gln Thr Ser Lys Arg His
            180                 185                 190

Gly Asp Lys Ser Phe Cys Pro Glu Ser Pro Gly Ile Leu Pro Arg Ser
        195                 200                 205

Ser Val Gly Pro Cys Ile Gln Ser Gln Leu Arg Lys Ser Arg Leu Gly
    210                 215                 220

Pro Gln Pro Ala Gln Gly Gln Leu Ala Gly Arg Gln Gln Gly Gly Ser
225                 230                 235                 240

Gly Ser Ile Arg Ala Arg Val His Pro Ser Ser Trp Gly Thr Val Gly
                245                 250                 255

Val Glu Pro Ser Gly Ser Gly Pro Thr His Asn Cys Ala Ser Ser Ser
            260                 265                 270

Ser Ser Cys Leu His Gln Ser Ala Val Arg Lys Ala Ala Tyr Ser Leu
        275                 280                 285

Ile Ser Thr Ser Lys Gly His Ser Ser Ser Gly His Ala Val Glu Leu
    290                 295                 300
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His His Phe Pro Pro Asn Ser Ser Arg Ser Gln Ser Gln Gly Pro Val
305                 310                 315                 320

Leu Ser Cys Trp Trp Leu Gln Phe Arg Asn Ser Glu Pro Cys Ser Glu
                325                 330                 335

Tyr Cys Leu Cys His Ile Val Asn Leu Ile Glu Asp Trp Gly Pro Cys
            340                 345                 350

Thr Glu His Gly Glu His Arg Ile Arg Thr Pro Arg Thr Pro Ala Arg
        355                 360                 365

Val Thr Gly Gly Val Phe Leu Val Asp Lys Asn Pro His Asn Thr Ala
    370                 375                 380

Glu Ser Arg Leu Val Val Asp Phe Ser Gln Phe Ser Arg Gly Asn Thr
385                 390                 395                 400

Arg Val Ser Trp Pro Lys Phe Ala Val Pro Asn Leu Gln Ser Leu Thr
                405                 410                 415

Asn Leu Leu Ser Ser Asn Leu Ser Trp Leu Ser Leu Asp Val Ser Ala
            420                 425                 430

Ala Phe Tyr His Leu Pro Leu His Pro Ala Ala Met Pro His Leu Leu
        435                 440                 445

Val Gly Ser Ser Gly Leu Ser Arg Tyr Val Ala Arg Leu Ser Ser Asn
    450                 455                 460

Ser Arg Ile Asn Asn Asn Glu His Arg Thr Met Glu Asn Leu His Asn
465                 470                 475                 480

Ser Cys Ser Arg Asn Leu Tyr Val Ser Leu Met Leu Leu Tyr Lys Thr
                485                 490                 495

Tyr Gly Gln Lys Leu His Leu Tyr Ser His Pro Ile Ile Leu Gly Phe
            500                 505                 510

Arg Lys Ile Pro Met Gly Val Gly Leu Ser Pro Phe Leu Leu Ala Gln
        515                 520                 525

Phe Thr Ser Ala Ile Cys Ser Val Val Arg Arg Ala Phe Pro His Cys
    530                 535                 540

Leu Ala Phe Ser Tyr Met Asp Asp Val Val Leu Gly Ala Lys Ser Val
545                 550                 555                 560

Gln His Leu Glu Ser Leu Tyr Ala Ala Val Thr Asn Phe Leu Leu Ser
                565                 570                 575

Leu Gly Ile His Leu Asn Pro His Lys Thr Lys Arg Trp Gly Tyr Ser
            580                 585                 590

Leu Asn Phe Met Gly Tyr Val Ile Gly Ser Trp Gly Thr Leu Pro Gln
        595                 600                 605
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Glu His Ile Val Gln Lys Ile Lys Met Cys Phe Arg Lys Leu Pro Val
    610                 615                 620

Asn Arg Pro Ile Asp Trp Lys Val Cys Gln Arg Ile Val Gly Leu Leu
625                 630                 635                 640

Gly Phe Ala Ala Pro Phe Thr Gln Cys Gly Tyr Pro Ala Leu Met Pro
                645                 650                 655

Leu Tyr Ala Cys Ile Gln Ala Lys Gln Ala Phe Thr Phe Ser Gln Thr
            660                 665                 670

Tyr Lys Thr Phe Leu Ser Lys Gln Tyr Leu Asn Leu Tyr Pro Val Ala
        675                 680                 685

Arg Gln Arg Pro Gly Leu Cys Glu Val Phe Ala Asp Ala Thr Pro Thr
    690                 695                 700

Gly Trp Gly Leu Ala Ile Gly His Gln Arg Met Arg Gly Thr Phe Val
705                 710                 715                 720

Ser Pro Leu Pro Ile His Thr Ala Glu Leu Leu Ala Ala Cys Phe Ala
                725                 730                 735

Arg Ser Arg Ser Gly Ala Lys Leu Ile Gly Thr Asp Asn Ser Val Val
            740                 745                 750

Leu Ser Arg Lys Tyr Thr Ser Phe Pro Trp Leu Leu Gly Cys Ala Ala
        755                 760                 765

Asn Trp Ile Leu Arg Gly Thr Ser Phe Val Tyr Val Pro Ser Ala Leu
    770                 775                 780

Asn Pro Ala Asp Asp Pro Ser Arg Gly Arg Leu Gly Leu Tyr Arg Pro
785                 790                 795                 800

Leu Leu Arg Leu Leu Tyr Arg Pro Thr Thr Gly Arg Thr Ser Leu Tyr
                805                 810                 815

Ala Asp Ser Pro Ser Val Pro Ser His Leu Pro Asp Arg Val His Phe
            820                 825                 830

Ala Ser Pro Leu His Val Ala Trp Arg Pro Pro
        835                 840
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 400 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Gly Gly Trp Ser Ser Lys Pro Arg Lys Gly Met Gly Thr Asn Leu
1               5                  10                  15

Ser Val Pro Asn Pro Leu Gly Phe Phe Pro Asp His Gln Leu Asp Pro
            20                  25                  30

Ala Phe Lys Ala Asn Ser Glu Asn Pro Asp Trp Asp Leu Asn Pro His
        35                  40                  45

Lys Asp Asn Trp Pro Asp Ala Asn Lys Val Gly Val Gly Ala Phe Gly
    50                  55                  60

Pro Gly Phe Thr Pro Pro His Gly Gly Leu Leu Gly Trp Ser Pro Gln
65                  70                  75                  80

Ala Gln Gly Leu Leu Thr Thr Val Pro Ala Ala Pro Pro Pro Ala Ser
                85                  90                  95

Thr Asn Arg Gln Ser Gly Arg Gln Pro Thr Pro Leu Ser Pro Pro Leu
            100                 105                 110

Arg Asp Thr His Pro Gln Ala Met Gln Trp Asn Ser Thr Thr Phe His
        115                 120                 125

Gln Thr Leu Gln Asp Pro Arg Val Arg Ala Leu Tyr Phe Pro Ala Gly
    130                 135                 140

Gly Ser Ser Ser Gly Thr Val Ser Pro Ala Gln Asn Thr Val Ser Ala
145                 150                 155                 160

Ile Ser Ser Ile Leu Ser Lys Thr Gly Asp Pro Val Pro Asn Met Glu
                165                 170                 175

Asn Ile Ala Ser Gly Leu Leu Gly Pro Leu Leu Val Leu Gln Ala Gly
            180                 185                 190

Phe Phe Leu Leu Thr Lys Ile Leu Thr Ile Pro Gln Ser Leu Asp Ser
        195                 200                 205

Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Pro Thr Val Cys Leu Gly
    210                 215                 220

Gln Asn Ser Gln Ser Gln Ile Ser Ser His Ser Pro Thr Cys Cys Pro
225                 230                 235                 240

Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile Ile
                245                 250                 255

Phe Leu Cys Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu
            260                 265                 270

Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser Thr
        275                 280                 285

Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln Gly
    290                 295                 300
```

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Thr Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Thr Asp Arg Asn
305                 310                 315                 320

Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys Tyr Leu
                325                 330                 335

Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val Pro
            340                 345                 350

Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser Val
        355                 360                 365

Ile Trp Met Met Trp Phe Trp Gly Pro Ser Leu Tyr Asn Ile Leu Ser
    370                 375                 380

Pro Phe Met Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr Ile
385                 390                 395                 400
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 212 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Gln Leu Phe His Leu Cys Leu Ile Ile Ser Cys Ser Cys Pro Thr
1               5                   10                  15

Val Gln Ala Ser Lys Leu Cys Leu Gly Trp Leu Trp Gly Met Asp Ile
            20                  25                  30

Asp Pro Tyr Lys Glu Phe Gly Ala Ser Val Glu Leu Leu Ser Phe Leu
        35                  40                  45

Pro Ser Asp Phe Phe Pro Ser Ile Arg Asp Leu Leu Asp Thr Ala Ser
    50                  55                  60

Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys Ser Pro His
65                  70                  75                  80

His Thr Ala Leu Arg Gln Ala Ile Leu Ser Trp Gly Glu Leu Met Asn
                85                  90                  95

Leu Ala Thr Trp Val Gly Ser Asn Leu Glu Asp Pro Ala Ser Arg Glu
            100                 105                 110

Leu Val Val Ser Tyr Val Asn Val Asn Met Gly Leu Lys Ile Arg Gln
        115                 120                 125

Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg Glu Thr Val
    130                 135                 140
```

```
Leu Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr Pro Pro Ala
145                 150                 155                 160

Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro Glu Thr Thr
                165                 170                 175

Val Val Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr Pro Ser Pro
            180                 185                 190

Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser Gln Ser Arg
        195                 200                 205

Glu Ser Gln Cys
    210
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 154 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Ala Ala Arg Leu Cys Cys Gln Leu Asp Pro Ala Arg Asp Val Leu
1               5                   10                  15

Cys Leu Arg Pro Val Gly Ala Glu Ser Arg Gly Arg Pro Leu Pro Gly
            20                  25                  30

Pro Leu Gly Ala Leu Pro Pro Ala Ser Pro Pro Val Ile Pro Thr Asp
        35                  40                  45

His Gly Ala His Leu Ser Leu Arg Gly Leu Pro Val Cys Ala Phe Ser
    50                  55                  60

Ser Ala Gly Pro Cys Ala Leu Arg Phe Thr Ser Ala Arg Arg Met Glu
65                  70                  75                  80

Thr Thr Val Asn Ala His Gly Asn Leu Pro Lys Val Leu His Lys Arg
                85                  90                  95

Thr Leu Gly Leu Ser Ala Met Ser Thr Thr Asp Leu Glu Ala Tyr Phe
            100                 105                 110

Lys Asp Cys Val Phe Asn Glu Trp Glu Glu Leu Gly Glu Glu Val Arg
        115                 120                 125

Leu Lys Val Phe Val Leu Gly Gly Cys Arg His Lys Leu Val Cys Ser
    130                 135                 140

Pro Ser Pro Cys Asn Phe Phe Thr Ser Ala
145                 150
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATAAGCTTATG CCCCTATCTT ATCAACACTT CCGGA 35

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAGTCTAGAC TCTGCGGTAT TGTGA 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAGTCTAGAC TCGTGGTGGA CTTCT 25

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGAGAATTCT CACGGTGGTC TCCATGCGAC GT 32

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TTTGTTTACG TCCCGT 16

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTAAGCTTAG TTTCCGGAAG TGTTGAT

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Chong Jin OON, et al.

Serial No.: 09/719,533 Group No.: --

Filed: December 13, 2000 Examiner: --

For: A VACCINE INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF

Attorney Docket No.: U 013108-9

Commissioner of Patents and Trademarks

Washington, D.C. 20231

SIRS:

PRELIMINARY AMENDMENT

Please amend the above identified application as follows:

IN THE CLAIMS

Please amend the following claims:

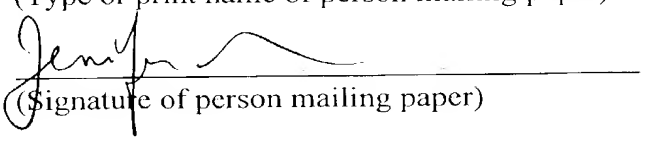
1. (Amended) An isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) and deposited under Accession Nos. P97121504, P97121505 and P97121506 with the European Collection of Cell Culture on 15th December 1997.

14. (Amended) The vector of claim 12, wherein the vector comprises viral DNA.

CERTIFICATE UNDER 37 1.10

I hereby certify that this paper is being deposited with the United States Postal Service on this date July 10, 2001 in an envelope as "EXPRESS MAIL POST OFFICE TO ADDRESSEE" Mailing Label Number EL728213866US addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231

JENNIFER RASHKIN
(Type or print name of person mailing paper)



(Signature of person mailing paper)

NOTE: Each paper or fee referred to as enclosed herein has the number of the "EXPRESS MAIL" mailing label place thereon prior to mailing 37 CFR 1.16(b).

21. (Amended) A purified polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121504, P97121505 and P97121506 with the European Collection of Cell Culture on 15th December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.

22. (Amended) A purified polypeptide obtained from a method which comprises:

(a) introducing a vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121504, P97121505 and P97121506 with the European Collection of Cell Culture on 15th December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine and operatively linked to a promoter of RNA transcription into a suitable host cell;

(b) culturing the resulting host cell so as to produce the polypeptide;

(c) recovering the polypeptide produced in step (b); and

(d) purifying the polypeptide so recovered.

24. (Amended) A purified peptide obtained from a method which comprises:

(a) introducing a vector comprising an isolated nuclei acid encoding a peptide which comprises at least a portion of a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121504, P97121505 and P97121506 with the European Collection of Cell Culture on 15th December 1997 wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1 into a suitable host cell;

(b) culturing the resulting host cell so as to produce the polypeptide;

(c) recovering the polypeptide produced in step (b); and

(d) purifying the polypeptide so recovered.

33. (Amended) The antibodies obtained in claim 27.

37. (Amended)A method for use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such method comprises

(a) obtaining an appropriate nucleic acid sample from the subject; and

(b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine.

38. (Amended) A method for use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such method comprises

(a) obtaining an appropriate nucleic acid sample from the subject; and

(b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface

antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;

(ii) isolating the complex so formed; and

(iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

39. (Amended) The method of claim 37, wherein the nucleic acid sample in step

(a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and

(ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from

the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, wherein the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

40. (Amended) The method of claim 37, wherein the determining of step (b) comprises:

(i) amplifying the nucleic acid present in the sample of step (a); and

(ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

41. (Amended) A method of use of antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such method comprises:

(a) obtaining an appropriate sample from the subject; and

(b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 so as to determine whether a subject is infected.

42. (Amended) The method of claim 37, wherein the isolated nucleic acid,

oligonucleotide, or antibody is labeled with a detectable marker.

43. (Amended) The method of claim 42, wherein the detectable marker is a radioactive isotope, a fluorophor, or an enzyme.

44. (Amended) The method of claim 37, wherein the sample comprises blood, tissue, or sera.

51. (Amended) A method comprising administering the composition of claim 47 for treating a subject infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).

52. (Amended) A method comprising administering the composition of claim 49 for treating a subject infected with a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).

53. (Amended) A method comprising administering the composition of claim 47 for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject.

54. (Amended) A method comprising administering the composition of claim 50 for preventing infection with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject.

57. (Amended) A method for use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein said method comprises:

(a) obtaining an appropriate nucleic acid sample from the subject; and

(b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid

sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.

58. (Amended) The method of claim 57, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) contacting the mRNA with an oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides within a nucleic acid which encodes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, without hybridizing to any sequence of nucleotides within a nucleic acid which encodes the major surface antigen of a wildtype hepatitis B virus under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;

(ii) isolating the complex so formed; and

(iii) identifying the mRNA in the isolated complex so as to thereby determined whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

59. (Amended) The method of claim 57, wherein the nucleic acid sample in step (a)

comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and

(ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, wherein the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

61. (Amended) A method for use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein said method comprises:

(a) obtaining an appropriate sample from the subject; and

(b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 36 so as to

determine whether the subject has a predisposition for hepatocellular carcinoma.

62. (Amended) The method of claim 58, wherein the oligonucleotide or antibody is labeled with a detectable marker.

69. (Amended) A method comprising administering the composition of claim 47 as a medicament for treating hepatocellular carcinoma.

70. (Amended) A method comprising administering the composition of claim 67 as a medicament for treating hepatocellular carcinoma.

71. (Amended) A method comprising administering the composition of claim 47 as a medicament for preventing hepatocellular carcinoma.

72. (Amended) A method comprising administering the composition of claim 67 as a medicament for preventing hepatocellular carcinoma.

IN THE SPECIFICATION

Please insert the following Sequence Listing on a separate page immediately after page 36:

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Oon, Chong Jin
Lim, Gek Keow
Leong, Ai Lin
Zhao, Yi
Chen, Wei Ning

(ii) TITLE OF INVENTION: A VACCINE-INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Ladas & Parry
(B) STREET: 26 West 61 Street
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: USA
(F) ZIP: 10023

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:09/719,533
(B) FILING DATE:13-DEC-2000
(C) CLASSIFICATION:435

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/SG98/00045
(B) FILING DATE: 19-JUN-1998

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Mass, Clifford J.
(B) REGISTRATION NUMBER: 30,086
(C) REFERENCE/DOCKET NUMBER:U-013108-9

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 708-1890

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3215 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTCCACCACT TTCCACCAAA CTCTTCAAGA TCCCAGAGTC AGGGCCCTGT ACTTTCCTGC 60

TGGTGGCTCC AGTTCAGGAA CAGTGAGCCC TGCTCAGAAT ACTGTCTCTG CCATATCGTC 120

AATCTTATCG AAGACTGGGG ACCCTGTACC GAACATGGAG AACATCGCAT CAGGACTCCT 180

AGGACCCCTG CTCGTGTTAC AGGCGGGGTT TTTCTTGTTG ACAAAAATCC TCACAATACC 240

GCAGAGTCTA GACTCGTGGT GGACTTCTCT CAATTTTCTA GGGGGAACAC CCGTGTGTCT 300

TGGCCAAAAT TCGCAGTCCC AAATCTCCAG TCACTCACCA ACCTGTTGTC CTCCAATTTG 360

TCCTGGTTAT CGCTGGATGT GTCTGCGGCG TTTTATCATC TTCCTCTGCA TCCTGCTGCT 420

ATGCCTCATC TTCTTGTTGG TTCTTCTGGA CTATCAAGGT ATGTTGCCCG TTTGTCCTCT 480

AATTCCAGGA TCAACAACAA CCAGCACCGG ACCATGCAAA ACCTGCACAA CTCCTGCTCA 540

AGGAACCTCT ATGTTTCCCT CATGTTGCTG TACAAAACCT ACGGACAGAA ACTGCACCTG 600

TATTCCCATC CCATCATCTT GGGCTTTCGC AAAATACCTA TGGGAGTGGG CCTCAGTCCG 660

TTTCTCTTGG CTCAGTTTAC TAGTGCCATT TGTTCAGTGG TTCGTAGGGC TTTCCCCCAC 720

TGTCTGGCTT TCAGTTATAT GGATGATGTG GTTTTGGGGG CCAAGTCTGT ACAACATCTT 780

GAGTCCCTTT ATGCCGCTGT TACCAATTTT CTTTTGTCTT TGGGTATACA TTTAAACCCT 840

CACAAAACAA AAAGATGGGG ATATTCCCTT AACTTCATGG GATATGTCAT TGGGAGTTGG 900

GGCACATTGC CACAGGAACA TATTGTACAA AAAATCAAAA TGTGTTTTAG GAAACTTCCT 960

GTAAACAGGC CTATTGATTG GAAAGTATGT CAACGAATTG TGGGTCTTTT GGGGTTTGCC 1020

GCCCCTTTCA CGCAATGTGG ATATCCTGCT TTAATGCCTT TATATGCATG TATACAAGCA 1080

AAACAGGCTT TTACTTTCTC GCAAACTTAC AAGACCTTTC TAAGTAAACA GTATCTGAAC 1140

CTTTACCCCG TTGCTCGGCA ACGCCCTGGT CTGTGCCAAG TGTTTGCTGA CGCAACCCCC 1200

ACTGGTTGGG GCTTGGCCAT AGGCCATCAG CGCATGCGTG GAACCTTTGT GTCTCCTCTG 1260

CCGATCCATA CTGCGGAACT CCTAGCCGCT TGTTTTGCTC GCAGCAGGTC TGGGGCAAAA 1320

CTCATCGGGA CTGACAATTC TGTCGTGCTC TCCCGCAAGT ATACATCATT TCCATGGCTG 1380

CTAGGCTGTG CTGCCAACTG GATCCTGCGC GGGACGTCCT TTGTTTACGT CCCGTCGGCG 1440

CTGAATCCCG CGGACGACCC CTCCCGGGGC CGCTTGGGGC TCTACCGCCC GCTTCTCCGC 1500

CTGTTATACC GACCGACCAC GGGGCGCACC TCTCTTTACG CGGACTCCCC GTCTGTGCCT 1560

TCTCATCTGC CGGACCGTGT GCACTTCGCT TCACCTCTGC ACGTCGCATG GAGACCACCG 1620

TGAACGCCCA CGGGAACCTG CCCAAGGTCT TGCATAAGAG GACTCTTGGA CTTTCAGCAA 1680

TGTCAACGAC CGACCTTGAG GCATACTTCA AAGACTGTGT GTTTAATGAG TGGGAGGAGT 1740

TGGGGGAGGA GGTTAGGTTA AAGGTCTTTG TACTAGGAGG CTGTAGGCAT AAATTGGTGT 1800

GTTCACCATC ACCATGCAAC TTTTTCACCT CTGCCTAATC ATCTCATGTT CATGTCCTAC 1860

TGTTCAAGCC TCCAAGCTGT GCCTTGGGTG GCTTTGGGGC ATGGACATTG ACCCGTATAA 1920

AGAATTTGGA GCTTCTGTGG AGTTACTCTC TTTTTTGCCT TCTGACTTTT TTCCTTCTAT 1980

TCGAGATCTC CTCGACACCG CCTCTGCTCT GTATCGGGAG GCCTTAGAGT CTCCGGAACA 2040

TTGTTCACCT CACCATACGG CACTCAGGCA AGCTATTCTG AGTTGGGGTG AGTTAATGAA 2100

TCTAGCCACC TGGGTGGGAA GTAATTTGGA AGATCCAGCA TCCAGGGAAT TAGTAGTCAG 2160

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CACTCCTCCT GCATATAGAC CACCAAATGC CCCTATCTTA TCAACACTTC CGGAAACTAC 2340

TGTTGTTAGA CGAAGAGGCA GGTCCCCTAG AAGAAGAACT CCCTCGCCTC GCAGACGAAG 2400

GTCTCAATCG CCGCGTCGCA GAAGATCTCA ATCTCGGGAA TCTCAATGTT AGTATTCCTT 2460

GGACACATAA GGTGGGAAAC TTTACGGGGC TTTATTCTTC TACGGTACCT TGCTTTAATC 2520

CTAAATGGCA AACTCCTTCT TTTCCGGACA TTCATTTGCA GGAGGACATT CTTGATAGAT 2580

GTAAGCAATT TGTGGGGCCC CTTACAGTAA ATGAAAACAG GAGACTAAAA TTAATTATGC 2640

CTGCTAGGTT TTATCCAAAT GTTACTAAAT ATTTGCCCTT AGATAAAGGG ATCAAACCAT 2700

ATTATCCAGA GTATGTAGTT AATCATTACT TCCAGACGCG ACATTATTTA CACACTCTTT 2760

GGAAGGCGGG GATCTTATAT AAAAGAGAGT CCACACGTAG CGCCTCATTT TGCGGGTCAC 2820

CATATTCTTG GGAACAAGAT CTACAGCATG GGAGGTTGGT CTTCCAAACC TCGAAAAGGC 2880

ATGGGGACAA ATCTTTCTGT CCCCAATCCC CTGGGATTCT TCCCCGATCA TCAGTTGGAC 2940

CCTGCATTCA AAGCCAACTC AGAAAATCCA GATTGGGACC TCAACCCGCA CAAGGACAAC 3000

TGGCCGGACG CCAACAAGGT GGGAGTGGGA GCATTCGGGC CAGGGTTCAC CCCTCCTCAT 3060

GGGGGACTGT TGGGGTGGAG CCCTCAGGCT CAGGGCCTAC TCACAACTGT GCCAGCAGCT 3120

CCTCCTCCTG CCTCCACCAA TCGGCAGTCA GGAAGGCAGC CTACTCCCTT ATCTCCACCT 3180

CTAAGGGACA CTCATCCTCA GGCCATGCAG TGGAA 3215

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 843 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Pro Leu Ser Tyr Gln His Phe Arg Lys Leu Leu Leu Leu Asp Glu
1 5 10 15

Glu Ala Gly Pro Leu Glu Glu Glu Leu Pro Arg Leu Ala Asp Glu Gly
20 25 30

Leu Asn Arg Arg Val Ala Glu Asp Leu Asn Leu Gly Asn Leu Asn Val
35 40 45

Ser Ile Pro Trp Thr His Lys Val Gly Asn Phe Thr Gly Leu Tyr Ser
50 55 60

Ser Thr Val Pro Cys Phe Asn Pro Lys Trp Gln Thr Pro Ser Phe Pro
65 70 75 80

Asp Ile His Leu Gln Glu Asp Ile Leu Asp Arg Cys Lys Gln Phe Val
85 90 95

Glu Pro Leu Thr Val Asn Glu Asn Arg Arg Leu Lys Leu Ile Met Pro
100 105 110

Ala Arg Phe Tyr Pro Asn Val Thr Lys Tyr Leu Pro Leu Asp Lys Gly
115 120 125

Ile Lys Pro Tyr Tyr Pro Glu Tyr Val Val Asn His Tyr Phe Gln Thr
130 135 140

Arg His Tyr Leu His Thr Leu Trp Lys Ala Gly Ile Leu Tyr Lys Arg
145 150 155 160

Glu Ser Thr Arg Ser Ala Ser Phe Cys Gly Ser Pro Tyr Ser Trp Glu
165 170 175

Gln Asp Leu Gln His Gly Arg Leu Val Phe Gln Thr Ser Lys Arg His
180 185 190

Gly Asp Lys Ser Phe Cys Pro Glu Ser Pro Gly Ile Leu Pro Arg Ser
195 200 205

Ser Val Gly Pro Cys Ile Gln Ser Gln Leu Arg Lys Ser Arg Leu Gly
210 215 220

Pro Gln Pro Ala Gln Gly Gln Leu Ala Gly Arg Gln Gln Gly Gly Ser
225 230 235 240

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Gly Ser Ile Arg Ala Arg Val His Pro Ser Ser Trp Gly Thr Val Gly
                  245                 250                 255

Val Glu Pro Ser Gly Ser Gly Pro Thr His Asn Cys Ala Ser Ser Ser
              260                 265                 270

Ser Ser Cys Leu His Gln Ser Ala Val Arg Lys Ala Ala Tyr Ser Leu
          275                 280                 285

Ile Ser Thr Ser Lys Gly His Ser Ser Ser Gly His Ala Val Glu Leu
      290                 295                 300

His His Phe Pro Pro Asn Ser Ser Arg Ser Gln Ser Gln Gly Pro Val
305                 310                 315                 320

Leu Ser Cys Trp Trp Leu Gln Phe Arg Asn Ser Glu Pro Cys Ser Glu
                  325                 330                 335

Tyr Cys Leu Cys His Ile Val Asn Leu Ile Glu Asp Trp Gly Pro Cys
              340                 345                 350

Thr Glu His Gly Glu His Arg Ile Arg Thr Pro Arg Thr Pro Ala Arg
          355                 360                 365

Val Thr Gly Gly Val Phe Leu Val Asp Lys Asn Pro His Asn Thr Ala
      370                 375                 380

Glu Ser Arg Leu Val Val Asp Phe Ser Gln Phe Ser Arg Gly Asn Thr
385                 390                 395                 400

Arg Val Ser Trp Pro Lys Phe Ala Val Pro Asn Leu Gln Ser Leu Thr
                  405                 410                 415

Asn Leu Leu Ser Ser Asn Leu Ser Trp Leu Ser Leu Asp Val Ser Ala
              420                 425                 430

Ala Phe Tyr His Leu Pro Leu His Pro Ala Ala Met Pro His Leu Leu
          435                 440                 445

Val Gly Ser Ser Gly Leu Ser Arg Tyr Val Ala Arg Leu Ser Ser Asn
      450                 455                 460

Ser Arg Ile Asn Asn Asn Glu His Arg Thr Met Glu Asn Leu His Asn
465                 470                 475                 480

Ser Cys Ser Arg Asn Leu Tyr Val Ser Leu Met Leu Leu Tyr Lys Thr
                  485                 490                 495

Tyr Gly Gln Lys Leu His Leu Tyr Ser His Pro Ile Ile Leu Gly Phe
              500                 505                 510

Arg Lys Ile Pro Met Gly Val Gly Leu Ser Pro Phe Leu Leu Ala Gln
          515                 520                 525

Phe Thr Ser Ala Ile Cys Ser Val Val Arg Arg Ala Phe Pro His Cys
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530 535 540

Leu Ala Phe Ser Tyr Met Asp Asp Val Val Leu Gly Ala Lys Ser Val
545 550 555 560

Gln His Leu Glu Ser Leu Tyr Ala Ala Val Thr Asn Phe Leu Leu Ser
565 570 575

Leu Gly Ile His Leu Asn Pro His Lys Thr Lys Arg Trp Gly Tyr Ser
580 585 590

Leu Asn Phe Met Gly Tyr Val Ile Gly Ser Trp Gly Thr Leu Pro Gln
595 600 605

Glu His Ile Val Gln Lys Ile Lys Met Cys Phe Arg Lys Leu Pro Val
610 615 620

Asn Arg Pro Ile Asp Trp Lys Val Cys Gln Arg Ile Val Gly Leu Leu
625 630 635 640

Gly Phe Ala Ala Pro Phe Thr Gln Cys Gly Tyr Pro Ala Leu Met Pro
645 650 655

Leu Tyr Ala Cys Ile Gln Ala Lys Gln Ala Phe Thr Phe Ser Gln Thr
660 665 670

Tyr Lys Thr Phe Leu Ser Lys Gln Tyr Leu Asn Leu Tyr Pro Val Ala
675 680 685

Arg Gln Arg Pro Gly Leu Cys Glu Val Phe Ala Asp Ala Thr Pro Thr
690 695 700

Gly Trp Gly Leu Ala Ile Gly His Gln Arg Met Arg Gly Thr Phe Val
705 710 715 720

Ser Pro Leu Pro Ile His Thr Ala Glu Leu Leu Ala Ala Cys Phe Ala
725 730 735

Arg Ser Arg Ser Gly Ala Lys Leu Ile Gly Thr Asp Asn Ser Val Val
740 745 750

Leu Ser Arg Lys Tyr Thr Ser Phe Pro Trp Leu Leu Gly Cys Ala Ala
755 760 765

Asn Trp Ile Leu Arg Gly Thr Ser Phe Val Tyr Val Pro Ser Ala Leu
770 775 780

Asn Pro Ala Asp Asp Pro Ser Arg Gly Arg Leu Gly Leu Tyr Arg Pro
785 790 795 800

Leu Leu Arg Leu Leu Tyr Arg Pro Thr Thr Gly Arg Thr Ser Leu Tyr
805 810 815

Ala Asp Ser Pro Ser Val Pro Ser His Leu Pro Asp Arg Val His Phe

820 825 830

Ala Ser Pro Leu His Val Ala Trp Arg Pro Pro
835 840

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 400 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Gly Gly Trp Ser Ser Lys Pro Arg Lys Gly Met Gly Thr Asn Leu
1 5 10 15

Ser Val Pro Asn Pro Leu Gly Phe Phe Pro Asp His Gln Leu Asp Pro
20 25 30

Ala Phe Lys Ala Asn Ser Glu Asn Pro Asp Trp Asp Leu Asn Pro His
35 40 45

Lys Asp Asn Trp Pro Asp Ala Asn Lys Val Gly Val Gly Ala Phe Gly
50 55 60

Pro Gly Phe Thr Pro Pro His Gly Gly Leu Leu Gly Trp Ser Pro Gln
65 70 75 80

Ala Gln Gly Leu Leu Thr Thr Val Pro Ala Ala Pro Pro Pro Ala Ser
85 90 95

Thr Asn Arg Gln Ser Gly Arg Gln Pro Thr Pro Leu Ser Pro Pro Leu
100 105 110

Arg Asp Thr His Pro Gln Ala Met Gln Trp Asn Ser Thr Thr Phe His
115 120 125

Gln Thr Leu Gln Asp Pro Arg Val Arg Ala Leu Tyr Phe Pro Ala Gly
130 135 140

Gly Ser Ser Ser Gly Thr Val Ser Pro Ala Gln Asn Thr Val Ser Ala
145 150 155 160

Ile Ser Ser Ile Leu Ser Lys Thr Gly Asp Pro Val Pro Asn Met Glu
165 170 175

Asn Ile Ala Ser Gly Leu Leu Gly Pro Leu Leu Val Leu Gln Ala Gly
180 185 190

Phe Phe Leu Leu Thr Lys Ile Leu Thr Ile Pro Gln Ser Leu Asp Ser
195 200 205

Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Pro Thr Val Cys Leu Gly
210 215 220

Gln Asn Ser Gln Ser Gln Ile Ser Ser His Ser Pro Thr Cys Cys Pro
225 230 235 240

Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile Ile
245 250 255

Phe Leu Cys Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu
260 265 270

Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser Thr
275 280 285

Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln Gly
290 295 300

Thr Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Thr Asp Arg Asn
305 310 315 320

Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys Tyr Leu
325 330 335

Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val Pro
340 345 350

Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser Val
355 360 365

Ile Trp Met Met Trp Phe Trp Gly Pro Ser Leu Tyr Asn Ile Leu Ser
370 375 380

Pro Phe Met Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr Ile
385 390 395 400

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 212 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Gln Leu Phe His Leu Cys Leu Ile Ile Ser Cys Ser Cys Pro Thr
1 5 10 15

Leu Trp Gly Met Asp Ile
30

Glu Leu Leu Ser Phe Leu
45

Leu Leu Asp Thr Ala Ser
60

Glu His Cys Ser Pro His
75 80

Trp Gly Glu Leu Met Asn
95

Asp Pro Ala Ser Arg Glu
110

Gly Leu Lys Ile Arg Gln
125

Phe Gly Arg Glu Thr Val
140

Ile Arg Thr Pro Pro Ala
155 160

Thr Leu Pro Glu Thr Thr
175

Arg Arg Thr Pro Ser Pro
190

Arg Arg Ser Gln Ser Arg
205

) Pro Ala Arg Asp Val Leu
15

ɜ Gly Arg Pro Leu Pro Gly
30

Pro Leu Gly Ala Leu Pro Pro Ala Ser P
35 40

His Gly Ala His Leu Ser Leu Arg Gly L
50 55

Ser Ala Gly Pro Cys Ala Leu Arg Phe T
65 70

Thr Thr Val Asn Ala His Gly Asn Leu P
85 9

Thr Leu Gly Leu Ser Ala Met Ser Thr T
100 105

Lys Asp Cys Val Phe Asn Glu Trp Glu G
115 120

Leu Lys Val Phe Val Leu Gly Gly Cys A
130 135

Pro Ser Pro Cys Asn Phe Phe Thr Ser A
145 150

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATAAGCTTATG CCCCTATCTT ATCAACACTT CCGGA

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7

GAGTCTAGAC TCTGCGGTAT TGTGA

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

```
Pro Leu Gly Ala Leu Pro Pro Ala Ser Pro Pro Val Ile Pro Thr Asp
        35                  40                  45

His Gly Ala His Leu Ser Leu Arg Gly Leu Pro Val Cys Ala Phe Ser
    50                  55                  60

Ser Ala Gly Pro Cys Ala Leu Arg Phe Thr Ser Ala Arg Arg Met Glu
65                  70                  75                  80

Thr Thr Val Asn Ala His Gly Asn Leu Pro Lys Val Leu His Lys Arg
                85                  90                  95

Thr Leu Gly Leu Ser Ala Met Ser Thr Thr Asp Leu Glu Ala Tyr Phe
            100                 105                 110

Lys Asp Cys Val Phe Asn Glu Trp Glu Glu Leu Gly Glu Glu Val Arg
        115                 120                 125

Leu Lys Val Phe Val Leu Gly Gly Cys Arg His Lys Leu Val Cys Ser
    130                 135                 140

Pro Ser Pro Cys Asn Phe Phe Thr Ser Ala
145                 150
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATAAGCTTATG CCCCTATCTT ATCAACACTT CCGGA 35

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAGTCTAGAC TCTGCGGTAT TGTGA 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAGTCTAGAC TCGTGGTGGA CTTCT 25

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGAGAATTCT CACGGTGGTC TCCATGCGAC GT 32

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TTTGTTTACG TCCCGT 16

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTAAGCTTAG TTTCCGGAAG TGTTGAT

REMARKS

The above amendatory action to the claims is taken solely for the purpose of avoiding claim fees that would otherwise accrue due to the presence of multiple dependent claims, with the exception that the Accession Nos. in claims 1, 21, 22 and 24 have been amended based on the disclosure in the Abstract of International Publication WO 99/66047 (PCT/SG98/00045) to correct an inadvertant clerical error.

The amendatory action to the specification is taken to comply with the applicable sequence listing requirements. A computer readable copy of the Sequence Listing and the requisite statement are submitted herewith.

Respectfully submitted,

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NEW YORK, NEW YORK 10023
REG. NO.: 30,086 (212) 708-1890

MARKED-UP COPY

1. (Amended) An isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) and deposited under Accession Nos. P971215014, P971215025 and P971215036 with the European Collection of Cell Culture on 15th December 1997.

14. (Amended) The vector of claim 12 or 13, wherein the vector comprises viral DNA.

21. (Amended) A purified polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P971215014, P971215025 and P971215036 with the European Collection of Cell Culture on 15th December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.

22. (Amended) A purified polypeptide obtained from a method which comprises:

(a) introducing a vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P971215014, P971215025 and P971215036 with the European Collection of Cell Culture on 15th December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine and operatively linked to a promoter of RNA transcription into a suitable host cell;

(b) culturing the resulting host cell so as to produce the polypeptide;

(c) recovering the polypeptide produced in step (b); and

(d) purifying the polypeptide so recovered.

24. (Amended) A purified peptide obtained from a method which comprises:

(a) introducing a vector comprising an isolated nuclei acid encoding a peptide which comprises at least a portion of a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P971215014, P971215025 and P971215036 with the European Collection of Cell Culture on 15th December 1997 wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1 into a suitable host cell;

(b) culturing the resulting host cell so as to produce the polypeptide;

(c) recovering the polypeptide produced in step (b); and

(d) purifying the polypeptide so recovered.

33. (Amended) The antibodies obtained in claim 27 or 31.

37. (Amended)A method for use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such determinationmethod comprises

(a) obtaining an appropriate nucleic acid sample from the subject; and

(b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine.

38. The use of claim 37(Amended) A method for use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a

major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such method comprises

(a) obtaining an appropriate nucleic acid sample from the subject; and

(b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;

(ii) isolating the complex so formed; and

(iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

39. (Amended) The usemethod of claim 37, wherein the nucleic acid sample in step

(a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the

amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and

(ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by thean isolated nucleic acid of claim 9encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, wherein the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

40. (Amended) The usemethod of claim 37, wherein the determining of step (b) comprises:

(i) amplifying the nucleic acid present in the sample of step (a); and

(ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

41. U(Amended) A method of use of antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of Hepantitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such determinationmethod comprises:

(a) obtaining an appropriate sample from the subject; and

(b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 or 36 so as to determine whether a subject is infected.

42. (Amended) The usemethod of claim 37, 38 or 41 wherein the isolated nucleic acid, oligonucleotide, or antibody is labeled with a detectable marker.

43. (Amended) The use themethod of claim 42, wherein the detectable marker is a radioactive isotope, a fluorophor, or an enzyme.

44. (Amended) The usemethod of claim 37, wherein the sample comprises blood, tissue, or sera.

51. Use of(Amended) A method comprising administering the composition of claim 47 or 48 for treating a subject infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).

52. Use of(Amended) A method comprising administering the composition of claim 49 for treating a subject infected with a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).

53. Use of(Amended) A method comprising administering the composition of claim 47 or 48 for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject.

54. Use of(Amended) A method comprising administering the composition of claim 50 for preventing infection with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject.

57. U(Amended) A method for use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determinationsaid method comprises:

(a) obtaining an appropriate nucleic acid sample from the subject; and

(b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.

58. (Amended) The method of claim 57, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) contacting the mRNA with thean oligonucleotide of claim 25 under conditionsat least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides within a nucleic acid which encodes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major

surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, without hybridizing to any sequence of nucleotides within a nucleic acid which encodes the major surface antigen of a wildtype hepatitis B virus under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;

(ii) isolating the complex so formed; and

(iii) identifying the mRNA in the isolated complex so as to thereby determined whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

59. (Amended) The method of claim 57, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and

(ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by thean isolated nucleic acid of claim 9encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, wherein the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

61. U(Amended) A method for use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determinationsaid method comprises:

(a) obtaining an appropriate sample from the subject; and

(b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 36 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.

62. (Amended) The method of claim 58, 59 or 60, wherein the oligonucleotide or antibody is labeled with a detectable marker.

69. Use of(Amended) A method comprising administering the composition of claim 47 or 48 as a medicament for treating hepatocellular carcinoma.

70. Use of(Amended) A method comprising administering the composition of claim 67 as a medicament for treating hepatocellular carcinoma.

71. Use of(Amended) A method comprising administering the composition of claim 47 or 48 as a medicament for preventing hepatocellular carcinoma.

72. Use of(Amended) A method comprising administering the composition of claim 67 as a medicament for preventing hepatocellular carcinoma.

A VACCINE-INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF

Throughout this application, various references are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains.

BACKGROUND OF THE INVENTION

The present invention concerns the hepatitis B virus genome with a vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) within the major surface antigen, its nucleotide sequence, the deduced four major protein sequences, antigen, antibody, detection systems, development of effective vaccines, and antiviral agents.

The hepatitis B virus was first discovered in 1963 as a human virus that is transmitted parenterally. Although these viruses are not particularly cytotoxic and do not lead to massive cell death, they have been the cause of a major infectious disease affecting both adults and young children worldwide. The presence of hepatitis B surface antigen has served as the main detection marker for carriers of hepatitis B virus, and thus, possibly those at risk of transmitting the virus. Conversely, the occurrence of an anti-surface antigen antibody indicates an immune response which would lead to eventual recovery. Stimulation of such immune response has been greatly helped by the currently licensed hepatitis B vaccines developed by Merck Sharpe & Dohme. These vaccines contain the major surface antigen in either the natural (plasma-derived) or the recombinant (purified from yeast cells) form, and have proven safe and effective in neutralizing the hepatitis B virus. In Singapore, the active vaccination program at a national scale has resulted in a significant decrease of acute hepatitis B infection and the incidence of primary hepatocellular carcinoma. This decrease has in turn been associated with an increased immunity in the population.

The major antigenic epitope of hepatitis B virus is a highly conserved region spanning 23 amino acid residues and located from amino acid position 124 to 147 of the major surface antigen. This small region designated as the group specific determinant "a" is found in all subtypes and isolates of hepatitis B viral genomes. Its antigenic properties seem due to its proposed double loop structure, to which the vaccine-induced neutralizing antibody binds.

In contrast to random mutations introduced into hepatitis B viral genomes during viral replication by the proof-reading defective reverse transcriptase, mutations induced following vaccination occur mainly in the "a" epitope of the major surface antigen. These mutant viruses are of particular interest since they show reduced affinity to the neutralizing antibody and therefore are able to replicate independently. Among these vaccine-escape mutants, the mutation at amino acid residue 145 (from Glycine to Arginine) in the second loop of the major surface antigen is the most significant because it is stable, results in conformational changes of the "a" epitope and has been reported worldwide in North America, Europe, Japan and Southeast Asia. In Singapore, for example, such mutants are the most frequent variant following vaccination. Twelve infectious variants among 41 breakthroughs have been identified as having an arginine mutation at amino acid residue 145 of the major surface antigen. There is evidence of vertical transmission from one of the 12 variants and this variant has also been associated with active liver disease. Significantly, some of these variants are now found in random asymptomatic adult population.

The occurrence of this replicative vaccine-induced mutant and its ability to escape detection using standard reagents is of grave concern because it has resulted in the development of acute hepatitis B in Italy and Singapore. This situation therefore requires the urgent development of specific detection systems, as well as, effective prophylactic

vaccines and antiviral agents. Determination of the nucleotide sequence of this vaccine-induced mutant virus constitutes the first step towards these aims and will certainly be helpful for the various above-mentioned developments.

SUMMARY OF THE INVENTION

This invention provides an isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) which constituent viral genome is deposited under Accession Nos. P97121504, P97121505 and P97121506 with the European Collection of Cell Culture on 15th December 1997.

This invention also provides an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.

This invention provides a method of producing the polypeptide in purified form and the resulting purified polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.

This invention provides an oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with sequences of only the mutant viral strain of hepatitis B virus.

This invention provides a method of obtaining antibodies to a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus and the antibodies produced.

This invention provides uses of the above-described polypeptide, antibodies or nucleic acid for determining whether a subject is infected with the above-described viral

strain.

This invention also provides a composition capable of stimulating or enhancing antibody production for the polypeptide.

This invention further provides a method for identifying a chemical compound which is capable of treating and/or preventing an infection by the above-described mutant viral strain and compositions containing such compounds.

This invention also provides a composition comprising the chemical compound identified by the above-described methods in an amount effective to treat or prevent infection by the strain and a pharmaceutically effective carrier.

This invention further provides use of compositions for treating a subject infected with this viral strain.

This invention also provides use of compositions for preventing infection of a subject by this viral strain.

And lastly, this invention provides a method of screening bodily fluids for this viral strain.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Structure of the four open reading frames of human hepatitis B viral genome isolated from a male, eleven year old Singaporean child with a glycine to arginine mutation at amino acid residue 145 of the major surface antigen, as labeled by an asterisk. The major viral proteins: DNA polymerase, large/middle/major surface antigen, precore, core and transactivating X are denoted as P, PreS 1/PreS2/S, PreC, C and X respectively.

Figure 2. Strategy of cloning and sequence determination of the same hepatitis B viral genome.

Figure 3. Whole nucleotide sequence of human hepatitis B virus, isolated from an eleven year old child born to a mother in Singapore with the wild type virus. The child had received standard Hepatitis B immunoglobulin (HBIG) and HB vaccine and was infected with the mutated strain one year later. This strain carries a mutation at amino acid residue 145 (glycine to arginine) of the major surface antigen (SEQ. I.D. No. 1). The mutation is shown at nucleic acids numbered 587-589.

Figure 4. Deduced amino acid sequence of the DNA polymerase from the nucleotide sequence of Figure 3 (SEQ. I.D. No. 2).

Figure 5. Deduced amino acid sequence of the large surface antigen from the nucleotide sequence of Figure 3. The mutated amino acid residue (G to R) is numbered 319 (SEQ. I.D. No. 3).

Figure 6. Deduced amino acid sequence of the core protein from the nucleotide sequence of Figure 3 (SEQ. I.D. No. 4).

Figure 7. Deduced amino acid sequence of the trans-activating X protein from the nucleotide sequence of Figure 3 (SEQ. I.D. No. 5).

Figure 8. Oligonucleotide seqence corresponding to the initiation site of the coding region of DNA polymerase, at position 2307 of the viral genome and matches the coding strand (sense oligonucleotide) (SEQ.I.D.No.6).

Figure 9. Oligonucleotide sequence corresponding to position 250 of the viral nucleotide sequence and matches the complementary strand (anti-sense oligonucleotide) (SEQ.I.D.No.7).

Figure 10. Oligonucleotide sequence corresponding to position 250 of the viral nucleotide sequence and matches the coding strand (sense oligonucleotide) (SEQ.I.D.No.8).

Figure 11. Oligonucleotide sequence corresponding to the stop codon of the coding region of DNA polymerase, at position 1623 of the viral genome and matches the complementary strand (anti-sense oligonucleotide) (SEQ.I.D.No.9).

Figure 12. Oligonucleotide sequence corresponding to position 1420 of the viral genome and matches the coding strand (sense oligonucleotide) (SEQ.I.D.No.10).

Figure 13. Oligonucleotide sequence corresponding to position 2340 of the viral genome and matches

the complementary strand (anti-sense oligonucleotide) (SEQ.I.D.No.11).

DETAILED DESCRIPTION OF THE INVENTION

Throughout this application, references to specific nucleotides are to nucleotides present on the coding strand of the nucleic acid. The following standard abbreviations are used throughout the specification to indicate specific nucleotides:

C=cytosine	A=adenosine
T=thymidine	G=guanosine

The present invention provides the nucleotide sequence of a hepatitis B virus genome, which carries a vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) of the major surface antigen, consisting of 3215 nucleotides (Figure 3) coding for 4 overlapping viral proteins shown in Figures 4-7.

The invention provides amino acid sequences of the four major viral proteins, these include the DNA polymerase, large/middle/major surface antigen, core and trans-activating X. These proteins can be produced using recombinant technology, and used in developing polyclonal or monoclonal antibodies.

The present invention also provides a hepatitis B virus diagnostic system, specific for the vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) of the major surface antigen, using nucleotide or protein sequences or antibodies described herein.

The present invention provides an isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) which constituent viral genome is deposited under Accession Nos. P97121504, P97121505 and P97121506.

The invention also provides an isolated nucleic acid encoding

a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of the major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine. In a specific embodiment, the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1, specifically, comprising nucleotides "AGA" in position 587-589, instead of "GGA." This nucleic acid can be DNA or RNA, specifically, cDNA or genomic DNA.

In another embodiment of the invention, the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.

This invention further provides an isolated nucleic acid which encodes a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.

This invention also provides an isolated nucleic acid which encodes a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.

This invention also provides a vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine and operatively linked to a promoter of RNA transcription.

Further, this invention provides a vector comprising an isolated nucleic acid encoding a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.

In both of the above-identified vectors, the vector may comprise viral DNA.

This invention also provides a host vector system for the production of a polypeptide which comprises the above-described vectors in a suitable host.

This invention also provides a method of producing a polypeptide or a peptide which comprises growing the host vector systems described above, under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.

This invention further provides a method of obtaining a polypeptide or a peptide in purified form which comprises: (a) introducing the above-described vectors into a suitable host cell; (b) culturing the resulting host cell so as to produce the polypeptide; (c) recovering the polypeptide produced in step (b); and (d) purifying the polypeptide so recovered.

This invention further provides a purified polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine. One means of obtaining the polypeptide is by the above-described method.

This invention also provides a purified peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence

designated Seq. I.D. No. 3. One means of obtaining this peptide is by the above-described method.

This invention also provides an oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides within a nucleic acid which encodes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, without hybridizing to any sequence of nucleotides within a nucleic acid which encodes the major surface antigen of a wild type hepatitis B virus. Specifically the oligonucleotide comprises nucleotides 527 through 595 of SEQ. I.D. No. 1.

This invention also provides a method of obtaining antibodies to a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and not to the major surface antigen of a wild type hepatitis B virus, comprising: (a) obtaining the polypeptide in a purified form; (b) immunizing an organism capable of producing antibodies against the purified polypeptide; (c) collecting the produced antibodies; (d) combining the produced antibodies and the purified polypeptide under conditions to form a complex; and (e) determining which produced antibodies form a complex with the purified polypeptide so as to obtain antibodies to the polypeptide. Specifically, the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1. In another embodiment, the polypeptide has an amino acid sequence substantially identical to amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.

One could perform the above-described well-known method in rabbits or mice.

This invention also provides a method of obtaining antibodies to a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3, comprising: (a) obtaining the peptide in a purified form; (b) immunizing an organism capable of producing antibodies against the purified peptide; (c) collecting the produced antibodies; (d) combining the produced antibodies and the purified peptide under conditions to form a complex; and (e) determining which produced antibodies form a complex with the purified peptide so as to obtain antibodies to the peptide.

One could perform the above-described well-known method in rabbits or mice.

This invention also provides the antibodies obtained from the above-described methods, specifically the monoclonal antibodies. Further, the invention provides antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and incapable of detecting the major surface antigen of a wild type hepatitis B virus, as well as, antibodies capable of detecting a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.

This invention also provides use of an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino

acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such determination comprises: (a) obtaining an appropriate nucleic acid sample from the subject; and (b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine.

A means of determination is where the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises: (i) contacting the mRNA with the above-described oligonucleotide under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex; (ii) isolating the complex so formed; and (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

Another example is where the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide

having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises: (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and (ii) comparing the amino acid sequence of step (i) with the amino acid sequence of an isolated nucleic acid which encodes a polypeptide, wherein the polypeptide has an amino acid sequence substantially identical to amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3 so as to thereby determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide. A further example is where the determining of step (b) comprises: (i) amplifying the nucleic acid present in the sample of step (a); and (ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

This invention provides the use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determination comprises: (a) obtaining an appropriate nucleic acid sample from the subject; and (b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.

This invention also provides use of antibodies capable of

detecting a polypeptide which is a mutant major surface antigen of a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such determination comprises: (a) obtaining an appropriate sample from the subject; and (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine by contacting the sample under appropriate conditions to bind to the antibodies so as to determine whether a subject is infected. Furthermore, the antibody may also be capable detecting a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298-320 of the amino acid sequence designated SEQ. I.D. No. 3.

In the above-described uses, the isolated nucleic acid, oligonucleotide or antibody may be labeled with a detectable marker. Examples of detectable markers include radioactive isotopes, fluorophors and enzymes.

In a specific embodiment, the sample includes, but is not limited to, blood, tissue or sera.

This invention also provides a method for identifying a chemical compound for the manufacture of a medicament which is capable of treating infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) which comprises: (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino

acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound; (b) detecting specific binding of the chemical compound to the polypeptide; and (c) determining whether the chemical compound inhibits the polypeptide so as to identify a chemical compound which is capable of treating infection by the viral strain.

This invention also provides a method for identifying a chemical compound for the manufacture of a medicament which is capable of preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), which comprises: (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound; (b) detecting specific binding of the chemical compound to the polypeptide; and (c) determining whether the chemical compound inhibits the polypeptide so as to identify a chemical compound which is capable of preventing infection by the viral strain.

This invention further provides a composition comprising a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, or derivative thereof, the amounts of such polypeptide being effective to stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable carrier.

The actual effective amount will be based upon the size of the polypeptide, the biodegradability of the polypeptide, the bioactivity of the polypeptide and the bioavailability of the polypeptide. If the polypeptide does not degrade quickly, is bioavailable and highly active, a smaller amount will be required to be effective. The effective amount will be known to one of skill in the art; it will also be dependent upon the form of the polypeptide, the size of the polypeptide and the bioactivity of the polypeptide. Use of an adjuvant for example, would lower the required amount of the polypeptide. One of skill in the art could routinely perform empirical activity tests to determine the bioactivity in bioassays and thus determine the effective amount.

Pharmaceutically acceptable carriers are well known to those skilled in the art and include, but are not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like.

This invention further provides a composition comprising a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3 or derivative thereof, the amounts of such peptide being effective to

stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable carrier.

This invention further provides compositions comprising the chemical compound identified by the above-described methods in an amount effective to treat or prevent infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) and a pharmaceutically effective carrier.

This invention provides the use of the above-described compositions as medicaments for treating and/or preventing hepatocellular carcinoma.

This invention also provides use of the above-identified compositions for treating a subject infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).

This invention also provides use of the above-identified compositions for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject which comprises administering an effective amount.

This invention further provides a method of screening tissues and bodily fluids from a subject for a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) which comprises: (a) obtaining an appropriate sample of bodily fluid from the subject; (b) determining the presence of a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine in the sample of step (a) so as to screen the sample for the strain. In embodiments of

this method, the bodily fluid comprises blood, sera, or a nucleic acid sample of blood or sera.

This invention provides a method for determining whether a subject has a predisposition for hepatocellular carcinoma, which comprises: (a) obtaining an appropriate nucleic acid sample from the subject; and (b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, thereby determining whether the subject has a predisposition for hepatocellular carcinoma.

This invention also provides the above-described method, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises: (i) contacting the mRNA with the above-described oligonucleotides under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex; (ii) isolating the complex so formed; and (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

This invention further provides the above-described method, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence

of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises: (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and (ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by the isolated nucleic acid described above so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

This invention also provides the above-described method, wherein the determining of step (b) comprises: (i) amplifying the nucleic acid present in the sample of step (a); and (ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

This invention further provides the above-described method for determining whether a subject has a predisposition for hepatocellular carcinoma, which comprises: (a) obtaining an appropriate sample from the subject; and (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the above-described antibodies so as to determine whether the subject has a predisposition for hepatocellular carcinoma.

This invention provides the above-described methods, wherein the oligonucleotide or antibody is labeled with a detectable marker.

This invention also provides the above-described methods,

wherein the detectable marker is a radioactive isotope, a fluorophor or an enzyme.

This invention also provides the above-described methods, wherein the sample comprises blood, tissue or sera.

This invention further provides a method for identifying a chemical compound for the manufacture of a medicament which is capable of treating hepatocellular carcinoma which comprises: (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound; (b) detecting specific binding of the chemical compound to the polypeptide; and (c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of treating hepatocellular carcinoma.

This invention provides a method for identifying a chemical compound for the manufacture of a medicament which is capable of preventing hepatocellular carcinoma, which comprises: (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound; (b) detecting specific binding of the chemical compound to the polypeptide; and (c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of preventing hepatocellular carcinoma.

Additionally, this invention provides a composition comprising the chemical compound identified by the above-described methods in an amount effective to treat hepatocellular carcinoma and a pharmaceutically effective carrier.

This invention also provides a composition comprising the chemical compound identified by the above-described methods in an amount effective to prevent hepatocellular carcinoma and a pharmaceutically effective carrier.

This invention further provides a method treating a subject with hepatocellular carcinoma which comprises administering an effective amount of the above-described compositions.

This invention further provides a method preventing hepatocellular carcinoma in a subject which comprises administering an effective amount of the above-described compositions.

This invention also provides a hepatitis vaccine, comprising a mutant form of the surface antigen of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of the major surface antigen of hepatitis B in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.

This invention also provides the above-described vaccine and an adjuvant.

This invention is illustrated in the Experimental Details section which follows. These sections are set forth to aid in an understanding of the invention but are not intended to, and should not be construed to, limit in any way the invention as set forth in the claims which follow thereafter.

<u>Experimental Details</u>

In the method described below, the hepatitis B virus carrying

the mutation at amino acid residue 145 of the major surface antigen was isolated, and its nucleotide sequence was determined.

Serum sample (20/815c) was obtained from an 11-year old child born to a mother carrying the wild type surface antigen. The child tested surface antigen negative at birth and was subjected to a combined treatment of hepatitis B immunoglobulin (HBIG) and plasma-derived vaccine. He was tested surface antigen positive one year later and carried an Arginine mutation at amino acid residue 145 in the major surface antigen. The viral DNA was extracted prior to the determination of its sequence in the present invention.

As described in the Examples below, the genome of this hepatitis B mutant virus carrying a mutation at amino acid residue 145 of the major surface antigen consists of 3215 nucleotides which are identical to those of the wild type virus of the same subtype (*adw*). Open reading frames (ORFs) coding for the major viral proteins are found at corresponding positions when compared to the wild type virus. Position 1 in the mutant hepatitis B virus genome is defined according to that in the wild type virus, corresponding to the restriction site EcoRI which is absent in the hepatitis B virus in the present invention due to changes in the nucleotide sequence.

The structure of the different ORFs in the mutant virus genome reported are summarized in Figure 1 and their locations are indicated as follows:

- DNA polymerase gene starts at position 2307 and ends at position 1623, therefore consisting of 2532 nucleotides and coding for 843 amino acid residues;
- Large surface antigen gene starts at position 2848 and ends at position 835, therefore consisting of 1203 nucleotides and coding for 400 amino acid residues. This large surface antigen overlaps the middle surface antigen starting at position 3205 and the major surface antigen which starts at position 155. Both the middle (consisting of 281 amino acids

residues) and the major (consisting of 226 amino acid residues) surface antigens end at the same position as the large surface antigen;
- Core gene starts at position 1814 and ends at position 2452, therefore consisting of 639 nucleotides and coding for 212 amino acid residues; and
- Trans-activating X gene starts at position 1374 and ends at position 1838, therefore consisting of 465 nucleotides and coding for 154 amino acid residues.

Furthermore, sequence analysis has established this mutant hepatitis B virus as belonging to adw subtype, as indicated by a lysine residue at both positions 122 and 160 in the major surface antigen. Consistent with previous analysis of the "a" epitope by direct sequencing, the vaccine-induced mutation (from Glycine to Arginine) is found at amino acid residue 145 of the major surface antigen.

Compared with the wild type hepatitis B virus deposited in the Genbank database (accession number D00329), the identity of this hepatitis B viral strain is at 89.4% for the nucleotide sequence. The identity of different viral proteins of the present mutant hepatitis B virus as compared with its wild type counterparts is at 88.3%,87.7%, 93.4% and 87% for DNA polymerase (PIR - Protein Identification Resources accession number P93460), large surface antigen (PIR accession number A93460), core (PIR accession number C93460) and trans-activating X (PIR accession number A31289) proteins, respectively.

The hepatitis B virus genome in the present invention carrying a vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) of the major surface antigen, can be used as material to design oligonucleotides specific to the mutant virus genome. These oligonucleotides can be used as material for highly specific diagnostic agents that detect virus carrying a vaccine-induced mutation at amino acid residue 145 of the major surface antigen.

The hepatitis B virus genome in the present invention, with a vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) of the major surface antigen, can be used as material to produce proteins of the invention by expressing a vector that carries the relevant coding region, and which can replicate in a host cell such as Escherichia coli, by standard DNA recombinant technology.

Proteins of the present invention are useful as material for highly specific diagnostic agents capable of detecting hepatitis B virus carrying a vaccine-induced mutation at amino acid residue 145 of the major surface antigen. Using known methods, these same proteins can be used to produce polyclonal and monoclonal antibodies.

Polyclonal and monoclonal antibodies can be used as material for diagnostic agents to detect with high specificity antigens of hepatitis B virus, with a vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) of the major surface antigen.

A detection system using each protein of the present invention or proteins with partial replacement of amino acids, and a detection system using monoclonal or polyclonal antibodies to such proteins, are useful as highly specific diagnostic agents for hepatitis B virus with vaccine-induced mutation at amino acid residue 145 of the major surface antigen, and are effective to detect and screen out such virus from transfusion bloods or blood derivatives. The proteins or antibodies to such proteins, can be used as a material for development of prophylactic and therapeutic vaccine against such virus.

It is well known that one or more nucleotides in a DNA sequence can be substituted by other nucleotides to produce the same protein. The present invention also concerns such nucleotide changes which code for proteins reported in this invention. It is equally well known that one or more amino acids in a protein sequence can be replaced by other analogous

amino acids, as defined by their hydrophilic properties or charges, to produce an analog of the amino acid sequence. Any analogs of the proteins of the present invention involving amino acid replacement, deletions, or by isosteres (modified amino acids that bear close structural and spatial similarity to protein amino acids), amino acid addition, or isostere addition can be utilized, provided that the resulting sequences elicit antibodies recognizing hepatitis B virus with a vaccine-induced mutation at amino acid mutation 145 (Glycine to Arginine) of the major surface antigen.

EXAMPLES

The nucleotide sequence and the deduced amino acid sequence of hepatitis B virus, carrying a vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) of the major surface antigen, were determined in the following manner:

1. Isolation of Viral DNA

The viral DNA was isolated from a serum sample (20/815c) obtained from the serum of an 11-year old Chinese child who was born to a mother of wild type hepatitis B virus surface antigen. Despite negative test results at birth, he was given combined treatment of HBIG and the plasma-derived vaccine and tested positive for hepatitis B virus surface antigen one year later. Sequence analysis of the "a" epitope indicated the presence of a vaccine-induced mutation at amino acid residue 145 of the major surface antigen.

The isolation method used was:
200 μl of the serum sample was added with 400 μl of the lysis buffer (Tris chloride 10 mM, pH7.4, EDTA 1 mM, and sodium dodecyl sulfate 2%) and 25 μl of proteinase K (20 mg/ml), incubated at 65 C for 3 hours. Viral DNA was then extracted by phenol/chloroform and precipitated by ethanol.

2. Amplification of Viral DNA by Polymerase Chain Reaction

(PCR)

The virus genome was amplified by polymerase chain reaction (PCR) using 3 sets of overlapping oligonucleotides, which were designed according to the wild type hepatitis B virus of adw subtype. Various restriction enzyme sites were included to facilitate the cloning of the PCR products. The position of these oligonucleotides is shown in Fig. 2 and indicated as follows:

- Flag 1 (ATAAGCTTATGCCCCTATCTTATCAACACTTCCGGA)(SEQ. I.D. No. 6) starts at the initiation site of the coding region of DNA polymerase, at position 2307 of the viral nucleotide sequence and matches the coding strand (sense oligonucleotide). An additional HindIII restriction enzyme site is underlined;
- Xba3 (GAGTCTAGACTCTGCGGTATTGTGA)(SEQ. I.D. No. 7) starts at the internal restriction enzyme site XbaI, at position 250 of the viral nucleotide sequence and matches the complementary strand (anti-sense oligonucleotide). An additional XbaI restriction enzyme site is underlined;
- Xba5 (GAGTCTAGACTCGTGGTGGACTTCT)(SEQ. I.D. No. 8) starts at the internal XbaI site, at the same location as that of Xba3 oligonucleotide but matches the coding strand (sense oligonucleotide). An additional XbaI restriction enzyme site is underlined;
- Common 3 (TGAGAATTCTCACGGTGGTCTCCATGCGACGT) (SEQ. I.D. No. 9) starts at the stop codon of the DNA polymerase, at position 1623 of the viral nucleotide sequence and matches the complementary strand (anti-sense oligonucleotide). An additional EcoRI restriction enzyme site is underlined;
- V11 (TTTGTTTACGTCCCGT)(SEQ. I.D. No. 10) starts near the initiation site of the X gene, at position 1420 of the viral nucleotide sequence and matches the coding strand (sense oligonucleotide);
- HindIIIADW3 (CTAAGCTTAGTTTCCGGAAGTGTTGAT) (SEQ. I.D. No. 11) starts close to the initiation site of the DNA polymerase, at position 2340 and matches the complementary strand (anti-sense oligonucleotide). An additional Hind III restriction enzyme site is underlined.

Using viral DNA as a template, PCR was then carried out on a DNA Thermal Cycler (Perkin-Elmer. Cetus) for 35 cycles using Pfu polymerase (Stratagene, U.S.A.), each cycle consisting of 1.5 minutes at a denaturing temperature of 94 °C, 2 minutes at an annealing temperature of 53 °C and 4 minutes at an extension temperature of 72 °C. The following combinations of oligonucleotides were used: Flag1/Xba3, Xba5/Common3 and V11/HindIIIADW3, and generating amplification products of 1.2 kb, 1.4 kb and 1.1 kb, respectively.

3. Cloning of the Amplified Viral DNA Fragments.

Amplified viral DNA fragment from Flag1/Xba3 (1.2 kb) was subjected to restriction enzyme digestion by HindIII and XbaI prior to cloning in a BlueScript plasmid pre-treated by the same restriction enzymes. Similar digestion with XbaI and EcoRI was applied to PCR product from Xba5/Common3 (1.4 kb) prior to cloning in a BlueScript plasmid pre-treated by XbaI and EcoRI. On the other hand, the DNA fragment amplified with V11 and HindIIIADW3 (1.1 kb) was directly cloned into ZeroBlunt plasmid, developed by InvitroGen (U.S.A.) for cloning blunt-end DNA fragments.

4. Determination of Nucleotide Sequence

Nucleotide sequence of the vaccine-induced hepatitis B virus in the present invention was determined on plasmid DNA template by chain-terminating inhibitors, using the Sequenase DNA Sequencing Kit (United States Biochemical Corp.). To facilitate the sequencing procedure, various internal oligonucleotides were designed (from V1 to V13) according to the wild type hepatitis B virus, and their positions are indicated in Fig. 2.

From the analysis described above, the full-length nucleotide sequence of the hepatitis B virus carrying a vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) of the major surface antigen was determined as shown in Figure 3.

The deduced amino acid sequences coding for the major viral proteins are shown in Figures 4-7: hepatitis B viral DNA polymerase (Figure 4), the large surface antigen (Figure 5), the core protein (Figure 6) and the trans-activating X protein (Figure 7).

Alignment of the virus sequence in the present invention with other hepatitis B viral sequences, available in the Genbank database, will point to specific sequence differences which in turn can be used to design DNA probes. A detection system using polymerase chain reaction (PCR) can then be developed. Such PCR reactions will involve combinations of oligonucleotides specific to hepatitis B virus with a vaccine-induced mutation at the amino acid residue 145 (Glycine to Arginine) of the major surface antigen, thereby allowing highly specific detection of these mutant viral DNAs. Briefly, viral DNA can be extracted as described in this invention. PCR reactions can be performed using specific oligonucleotides using similar cycling conditions described above. Results can then be analyzed after resolving PCR products on a 1 % agarose gel.

According to known immunological procedures, it is possible to determine epitopes from protein sequences such as those in Figures 4-7. Determination of these epitopes specific to hepatitis B virus with vaccine-induced mutation at amino acid residue 145 of the major surface antigen will allow the synthesis of peptides using genetic engineering methods, synthesis of the proteins, production of the antibodies, development of specific diagnostic reagents, development of prophylactic and therapeutic vaccines, and antiviral agents.

A detection system for antibodies against hepatitis B virus, with a vaccine-induced mutation at amino acid residue 145 of the major surface antigen, can be developed using polyvinyl microtiter plates and the sandwich method. Briefly, 50 μl of 5 μg/ml concentration of a hepatitis B virus (vaccine-induced mutant) peptide can be dispensed in each well of the

microtiter plates and incubated overnight at room temperature for consolidation. Similar procedures can be applied to 's' protein purified from host cells such as Escherichia coli. The microplate wells can be washed five times with physiological saline solution containing 0.05% Tween 20. For overcoating, 100 μl of NaCl buffer containing 30% (v/v) of calf serum and 0.05% Tween 20 (CS buffer) can be dispensed in each well and discarded after incubation for 30 minutes at room temperature.

To determine antibodies specific for the vaccine-induced mutant hepatitis B virus antibodies in serum, the primary reaction can be carried out such that 50 μl of the CS buffer and 10 μl of a serum sample can be dispensed in each microplate well and incubated on a microplate vibrator for one hour at room temperature. After completion of the primary reaction, microplate wells are washed five times as described above.

In the secondary reaction, 1 ng of horseradish peroxidase labeled anti-human IgG mouse monoclonal antibodies dissolved in 50 μl of calf serum can be dispensed in each microplate well, and incubated on a microplate vibrator for one hour at room temperature. Upon completion, wells can be washed five times in the same way. After addition of hydrogen peroxide (as substrate) and 50 μl of O-phenylendiamine solution (as color developer) in each well, and after incubation for 30 minutes at room temperature, 50 μl of 4M sulphuric acid solution can be dispensed in each well to stop further color development and for reading absorbance at 490nm.

The present invention makes possible detection of vaccine-induced mutant hepatitis B virus, in particular those carrying a mutation at amino acid residue 145 of the major surface antigen. Such mutant hepatitis B virus have hitherto escaped the detection using conventional antibody-based methods, and the present invention also provides detection systems capable of highly specific and sensitive detection at

an early stage of infection.

In addition, these features allow accurate diagnosis of patients at an early stage of the disease and also help to remove with higher efficiency blood contaminated with vaccine-induced mutant hepatitis B virus through using a screening test of donor bloods.

Proteins and their antibodies under the present invention can be utilized for development of prophylactic and therapeutic vaccines, as well as, immunological pharmaceuticals. Sequence information on structural genes of these mutant viruses will be helpful in developing detection systems of the relevant protein antigens and antibodies.

Antigen-antibody complexes can be detected by known methods. Specific monoclonal and polyclonal antibodies can be raised by immunizing animals such as mice and rabbits with peptides or proteins specific to mutant vaccine-induced hepatitis B viruses. Inhibitory antiviral agents can be designed and targeted against these proteins and molecules in cell culture or in vivo.

The present invention is based on studies on an isolated virus genome with a vaccine-induced mutation at amino acid residue 145 (Glycine to Arginine) of the major surface antigen. The invention makes possible highly specific detection of these vaccine-induced mutant hepatitis B virus and provides material such as protein, polyclonal and monoclonal antibodies for development of such detection system.

We have evidence showing that when expressed in a mammalian expression system, the major surface antigen (HBsAg) of the mutant HBV reported in our invention is detected as a 22kDa protein on a Coomassie-blue stained SDS-PAGE gel, whereas the wild type HBsAg is detected as a 25kDa protein. Since the only glycosylation site on the HBsAg is located in close proximity (Asparagine at position 146) to the mutation at

position 145, it is very likely that the drastic change from Glycine to Arginine at position 145 affects the glycosylation process in the mutant HBsAg. This defective glycosylation process would in turn result in the smaller protein as observed in our studies. As the antigenicity of a protein is adversely affected by the extent of its glycosylation, we predict that the mutant HBsAg reported in our invention is more antigenic than the wild type HBsAg. This prediction is further supported by the structural analysis using the method developed by Hopp and Woods.

Our serological studies also indicate major differences between wild type HBV carriers and those carrying the mutant HBV reported in our invention, one such difference is that the serum viral DNA load is significantly lower at around 5pg per ml as measured by an ^{125}I hybridization assay (Abbott Laboratories, U.S.A.). In marked contrast, the wild type HBV DNA load is much higher at over 100pg per ml. In addition, the anti-Hepatitis B surface antigen (anti-HBs) level is detectable at 10 IU per ml in the mutant HBV reported in our invention, whereas such antibodies remained undetectable in carriers of wild type HBV.

In the process of sequence determination, we have observed that a few oligonucleotides (each of them longer than 17 bases) designed according to the wild type HBV sequence, failed to hybridize to the mutant viral DNA reported in our invention. Two such oligonucleotides are located in the genome at positions 2711 to 2729 and 2902 to 2920. Our results, therefore, point to the structural differences between the wild type and mutant HBV genomes.

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What is claimed is:

1. An isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) and deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15th December 1997.

2. An isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.

3. The isolated nucleic acid of claim 2, wherein the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1.

4. The isolated nucleic acid of claim 3, comprising nucleotides "AGA" in positions 587-589.

5. The isolated nucleic acid of claim 2, wherein the nucleic acid is DNA.

6. The isolated nucleic acid of claim 2, wherein the nucleic acid is RNA.

7. The isolated nucleic acid of claim 5, wherein the nucleic acid is cDNA.

8. The isolated nucleic acid of claim 5, wherein the nucleic acid is genomic DNA.

9. The isolated nucleic acid of claim 2, wherein the polypeptide

has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.

10. An isolated nucleic acid which encodes a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.

11. An isolated nucleic acid which encodes a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.

12. A vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine and operatively linked to a promoter of RNA transcription.

13. A vector comprising an isolated nucleic acid encoding a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.

14. The vector of claim 12 or 13, wherein the vector comprises viral DNA.

15. A host vector system for the production of a polypeptide which comprises the vector of claim 12 in a suitable host.

16. A host vector system for the production of a peptide which comprises the vector of claim 13 in a suitable host.

17. A method of producing a polypeptide which comprises growing the host vector system of claim 15 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.

18. A method of producing a peptide which comprises growing the host vector system of claim 16 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.

19. A method of obtaining a polypeptide in purified form which comprises:
    (a) introducing the vector of claim 12 into a suitable host cell;
    (b) culturing the resulting host cell so as to produce the polypeptide;
    (c) recovering the polypeptide produced in step (b); and
    (d) purifying the polypeptide so recovered.

20. A method of obtaining a polypeptide in purified form which comprises:
    (a) introducing the vector of claim 13 into a suitable host cell;
    (b) culturing the resulting host cell so as to produce the polypeptide;
    (c) recovering the polypeptide produced in step (b); and
    (d) purifying the polypeptide so recovered.

21. A purified polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15th December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.

22. A purified polypeptide obtained from a method which comprises:
    (a) introducing a vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15th December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine and operatively linked to a promoter of RNA transcription into a suitable host cell;
    (b) culturing the resulting host cell so as to produce the polypeptide;
    (c) recovering the polypeptide produced in step (b) ; and
    (d) purifying the polypeptide so recovered.

23. A purified peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.

24. A purified peptide obtained from a method which comprises:
    (a) introducing a vector comprising an isolated nuclei acid encoding a peptide which comprises at least a portion of a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15th December 1997 wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1 into a suitable host cell;
    (b) culturing the resulting host cell so as to produce the polypeptide;
    (c) recovering the polypeptide produced in step (b); and

(d) purifying the polypeptide so recovered.

25. An oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides within a nucleic acid which encodes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, without hybridizing to any sequence of nucleotides within a nucleic acid which encodes the major surface antigen of a wildtype hepatitis B virus.

26. The oligonucleotide of claim 25 comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.

27. A method of obtaining antibodies to a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and not to the major surface antigen of a wildtype hepatitis B virus, comprising:
(a) obtaining the polypeptide in a purified form;
(b) immunizing an organism capable of producing antibodies against the purified polypeptide;
(c) collecting the produced antibodies;
(d) combining the produced antibodies and the purified polypeptide under conditions to form a complex; and
(e) determining which produced antibodies form a complex with the purified polypeptide so as to obtain antibodies to the polypeptide.

28. The method of claim 27, wherein the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1.

29. The method of claim 27, wherein the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.

30. The method of claim 27, wherein the organism comprises a rabbit or a mouse.

31. A method of obtaining antibodies to a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3, comprising:
(a) obtaining the peptide in a purified form;
(b) immunizing an organism capable of producing antibodies against the purified peptide;
(c) collecting the produced antibodies;
(d) combining the produced antibodies and the purified peptide under conditions to form a complex; and
(e) determining which produced antibodies form a complex with the purified peptide so as to obtain antibodies to the peptide.

32. The method of claim 31, wherein the organism comprises a rabbit or a mouse.

33. The antibodies obtained in claim 27 or 31.

34. Monoclonal antibodies of the antibodies of claim 33.

35. Antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus,

such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and incapable of detecting the major surface antigen of a wildtype hepatitis B virus.

36. Antibodies capable of detecting a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.

37. Use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such determination comprises

(a) obtaining an appropriate nucleic acid sample from the subject; and

(b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine.

38. The use of claim 37, wherein the nucleic acid sample in step

(a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;

(ii) isolating the complex so formed; and

(iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

39. The use of claim 37, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and

(ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by the isolated nucleic acid of claim 9 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

40. The use of claim 37, wherein the determining of step (b) comprises:

(i) amplifying the nucleic acid present in the sample of step (a); and

(ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

41. Use of antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of Hepantitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such determination comprises:

(a) obtaining an appropriate sample from the subject; and

(b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 or 36 so as to determine whether a subject is infected.

42. The use of claim 37, 38 or 41, wherein the isolated nucleic acid, oligonucleotide, or antibody is labeled with a detectable marker.

43. The use the claim 42, wherein the detectable marker is a radioactive isotope, a fluorophor, or an enzyme.

44. The use of claim 37, wherein the sample comprises blood, tissue, or sera.

45. A method for identifying a chemical compound for use in the manufacture of a medicament capable of treating infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein the method for identifying the chemical compound comprises:

(a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;

(b) detecting specific binding of the chemical compound to the polypeptide; and

(c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of treating infection by the viral strain.

46. A method for identifying a chemical compound for use in the manufacture of a medicament capable of preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein the method for identifying the chemical compound comprises:

(a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen

of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;

(b) detecting specific binding of the chemical compound to the polypeptide; and

(c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of preventing infection by the viral strain.

47. A composition comprising a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15th December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, the amounts of such polypeptide being effective to stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable carrier.

48. A composition comprising a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3., the amounts of such peptide being effective to stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable carrier.

49. A method of obtaining a composition which comprises a identifying a chemical compound by the method of claim 45 and admixing the compound with a pharmaceutically effective carrier.

50. A method of obtaining a composition which comprises a identifying a chemical compound by the method of claim 46 and admixing the compound with a pharmaceutically effective carrier.

51. Use of the composition of claim 47 or 48 for treating a subject infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).

52. Use of the composition of claim 49 for treating a subject infected with a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).

53. Use of the composition of claim 47 or 48 for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject.

54. Use of the composition of claim 50 for preventing infection with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject.

55. A method of screening bodily fluids from a subject for a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) which comprises:

(a) obtaining an appropriate sample of bodily fluid from the subject;

(b) determining the presence of a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major

surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, in the sample of step (a) so as to screen the sample for the strain.

56. The method of claim 55, wherein the bodily fluid comprises blood, sera, or a nucleic acid sample of blood or sera.

57. Use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determination comprises:

(a) obtaining an appropriate nucleic acid sample from the subject; and

(b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.

58. The method of claim 57, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a

glycine, and wherein the determining of step (b) comprises:

(i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;

(ii) isolating the complex so formed; and

(iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

59. The method of claim 57, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

(i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and

(ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by the isolated nucleic acid of claim 9 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

60. The method of claim 57, wherein the determining of step (b) comprises:

(i) amplifying the nucleic acid present in the sample of step (a); and

(ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

61. Use of an antibody that recognizes a polypeptide which is a

mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determination comprises:

(a) obtaining an appropriate sample from the subject; and

(b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 36 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.

62. The method of claim 58, 59 or 60, wherein the oligonucleotide or antibody is labeled with a detectable marker.

63. The method of claim 62, wherein the detectable marker is a radioactive isotope, a fluorophor or an enzyme.

64. The method of claim 57, wherein the sample comprises blood, tissue or sera.

65. A method for identifying a chemical compound for use in the manufacture of a medicament capable of treating hepatocellular carcinoma wherein the method for identifying the chemical compound comprises:

(a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen

of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;

(b) detecting specific binding of the chemical compound to the polypeptide; and

(c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of treating hepatocellular carcinoma.

66. A method for identifying a chemical compound for use in the manufacture of a medicament capable of preventing hepatocellular carcinoma, wherein the method for identifying the chemical compound comprises:

(a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;

(b) detecting specific binding of the chemical compound to the polypeptide; and

(c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of preventing hepatocellular carcinoma.

67. A method of obtaining a composition which comprises a identifying a chemical compound by the method of claim 65 and admixing the compound with a pharmaceutically effective carrier.

68. A method of obtaining a composition which comprises a identifying a chemical compound by the method of claim 66 and admixing the compound with a pharmaceutically effective carrier.

69. Use of the composition of claim 47 or 48 as a medicament for treating hepatocellular carcinoma.

70. Use of the composition of claim 67 as a medicament for treating hepatocellular carcinoma.

71. Use of the composition of claim 47 or 48 as a medicament for preventing hepatocellular carcinoma.

72. Use of the composition of claim 67 as a medicament for preventing hepatocellular carcinoma.

73. A hepatitis vaccine, comprising a mutant form of the surface antigen of hepatitis B virus deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15th December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of the major surface antigen of hepatitis B in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.

74. The vaccine of claim 73, further comprising an adjuvant.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :		
C12N 15/51, 7/01, C07K 14/02, 16/08, C12Q 1/68, A61K 39/29	**A1**	**(11) International Publication Number:** **WO 99/66047** **(43) International Publication Date:** 23 December 1999 (23.12.99)

(21) International Application Number: PCT/SG98/00045

(22) International Filing Date: 19 June 1998 (19.06.98)

(71) Applicant *(for all designated States except US)*: GOVERNMENT OF THE REPUBLIC OF SINGAPORE [SG/SG]; Ministry of Health, College of Medicine Building, 18 College Road, Singapore 169854 (SG).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: OON, Chong, Jin [SG/SG]; 14A Princess of Wales Road, Singapore 266 914 (SG). LIM, Gek, Keow [SG/SG]; 16 Telok Kurau, Lorong G, Singapore 426180 (SG). LEONG, Ai, Lin [SG/SG]; Block 263, Yishun Street 22 #12-161, Singapore 760263 (SG). ZHAO, Yi [SG/SG]; Block 345, Bukit Batok Street 34 #12-270, Singapore 650345 (SG). CHEN, Wei, Ning [SG/SG]; Block 104, Spottiswoode Park Road #22-114, Singapore 080104 (SG).

(74) Agent: DREW & NAPIER; 20 Raffles Place, #17-00 Ocean Towers, Singapore 048620 (SG).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: A VACCINE-INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF

(57) Abstract

This invention provides an isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-´S-145 Singapore Strain (Glycine to Arginine) which constituent viral genome is deposited under Accession Nos. P97121504, P97121505 and P97121506 with the European Collection of Cell Culture on 15th December 1997. This invention also provides an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wild type hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, and the purified peptide. This invention further provides an isolated nucleic acid which encodes a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. ID. No. 1, and the purified peptide. This invention also provides various methods using the disclosed isolated nucleic acids and polypeptides. This invention also provides various uses of the viral strain and its proteins.

#3

Practitioner's Docket No. U013108-9 ***PATENT***

Optional Customer No. Bar Code

00140

PATENT TRADEMARK OFFICE

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION, OR C-I-P)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type:

(check one applicable item below)

[ ] original.
[ ] design.

NOTE: With the exception of a supplemental oath or declaration submitted in a reissue, a supplemental oath or declaration is not treated as an amendment under 37 CFR 1.312 (Amendments after allowance). M.P.E.P. Section 714.16, 7th Ed.

[ ] supplemental.

NOTE: If the declaration is for an International Application being filed as a divisional, continuation or continuation-in-part application, do not check next item; check appropriate one of last three items.

[x] national stage of PCT.

NOTE: If one of the following 3 items apply, then complete and also attach ADDED PAGES FOR DIVISIONAL, CONTINUATION OR C-I-P.

NOTE: See 37 C.F.R. Section 1.63(d) (continued prosecution application) for use of a prior nonprovisional application declaration in the continuation or divisional application being filed on behalf of the same or fewer of the inventors named in the prior application.

[ ] divisional.
[ ] continuation.

NOTE: Where an application discloses and claims subject matter not disclosed in the prior application, or a continuation or divisional application names an inventor not named in the prior application, a continuation-in-part application must be filed under 37 C.F.R. Section 1.53(b) (application filing requirements-nonprovisional application).

[ ] continuation-in-part (C-I-P).

INVENTORSHIP IDENTIFICATION

WARNING: *If the inventors are each not the inventors of all the claims, an explanation of the facts, including the ownership of all the claims at the time the last claimed invention was made, should be submitted.*

My residence, post office address and citizenship are as stated below, next to my name. I believe that I am the original, first and sole inventor *(if only one name is listed below)* or an original, first and joint inventor *(if plural names are listed below)* of the subject matter that is claimed, and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

A VACCINE-INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF

SPECIFICATION IDENTIFICATION

The specification of which:

(complete (a), (b), or (c))

(a) [ ] is attached hereto.

NOTE: *"The following combinations of information supplied in an oath or declaration filed on the application filing date with a specification are acceptable as minimums for identifying a specification and compliance with any one of the items below will be accepted as complying with the identification requirement of 37 C.F.R. Section 1.63:*

"(1) name of inventor(s), and reference to an attached specification which is both attached to the oath or declaration at the time of execution and submitted with the oath or declaration on filing;

"(2) name of inventor(s), and attorney docket number which was on the specification as filed; or

"(3) name of inventor(s), and title which was on the specification as filed."

Notice of July 13, 1995 (1177 O.G. 60).

(b) [X] was filed on December 13, 2000 [ ] as Application No. 09/719,533
[ ] and was amended on _____ (if applicable).

NOTE: *Amendments filed after the original papers are deposited with the PTO that contain new matter are not accorded a filing date by being referred to in the declaration. Accordingly, the amendments involved are those filed with the application papers or, in the case of a supplemental declaration, are those amendments claiming matter not encompassed in the original statement of invention or claims. See 37 C.F.R. Section 1.67.*

NOTE: *"The following combinations of information supplied in an oath or declaration filed after the filing date are acceptable as minimums for identifying a specification and compliance with any one of the items below will be accepted as complying with the identification requirement of 37 C.F.R. Section 1.63:*

(A) application number (consisting of the series code and the serial number, e.g., 08/123,456);
(B) serial number and filing date;
(C) attorney docket number which was on the specification as filed;
(D) title which was on the specification as filed and reference to an attached specification which is both attached to the oath or declaration at the time of execution and submitted with the oath or declaration; or
(E) title which was on the specification as filed and accompanied by a cover letter accurately identifying the application for which it was intended by either the application number (consisting of the series code and the serial number, e.g., 08/123,456), or serial number and filing date. Absent any statement(s) to the contrary, it will be presumed that the application filed in the PTO is the application which the inventor(s) executed by signing the oath or declaration.

M.P.E.P. Section 601.01(a), 7th ed.

(c) [x] was described and claimed in PCT International Application No. PCT/SG98/00045 filed on June 19, 1998 and as amended under PCT Article 19 on _____ *(if any)*.

SUPPLEMENTAL DECLARATION (37 C.F.R. Section 1.67(b))

(complete the following where a supplemental declaration is being submitted)

[ ] I hereby declare that the subject matter of the

[ ] attached amendment
[ ] amendment filed on _____.

was part of my/our invention and was invented before the filing date of the original application, above identified, for such invention.

ACKNOWLEDGMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in 37, Code of Federal Regulations, Section 1.56,

(also check the following items, if desired)

[ ] and which is material to the examination of this application, namely, information where there is a substantial likelihood that a reasonable Examiner would consider it important in deciding whether to allow the application to issue as a patent, and

[ ] in compliance with this duty, there is attached an information disclosure statement, in accordance with 37 C.F.R. Section 1.98.

PRIORITY CLAIM (35 U.S.C. Section 119(a)-(d))

NOTE: "The claim to priority need be in no special form and may be made by the attorney or agent if the foreign application is referred to in the oath or declaration as required by Section 1.63. The claim for priority and the certified copy of the foreign application specified in 35 U.S.C. Section 119(b) must be filed in the case of an interference (Section 1.630), when necessary to overcome the date of a reference relied upon by the examiner, when specifically required by the examiner, and in all other situations, before the patent is granted. If the claim for priority or the certified copy of the foreign application is filed after the date the issue fee is paid, it must be accompanied by a petition requesting entry and by the fee set forth in Section 1.17(i). If the certified copy is not in the English language, a translation need not be filed except in the case of interference; or when necessary to overcome the date of a reference relied upon by the examiner; or when specifically required by the examiner, in which event an English language translation must be filed together with a statement that the translation of the certified copy is accurate." 37 C.F.R. Section 1.55(a).

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

(complete (d) or (e))

(d) [x] no such applications have been filed.
(e) [ ] such applications have been filed as follows.

NOTE: Where item (c) is entered above and the International Application which designated the U.S. itself claimed priority check item (e), enter the details below and make the priority claim.

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. SECTION 119(a)-(d)

COUNTRY (OR INDICATE IF PCT)	APPLICATION NUMBER	DATE OF FILING DAY, **MONTH**, YEAR	PRIORITY CLAIMED UNDER 35 USC 119
			[ ]YES [ ]NO
			[ ]YES [ ]NO
			[ ]YES [ ]NO
			[ ]YES [ ]NO
			[ ]YES [ ]NO

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
(35 U.S.C. Section 119(e))

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below:

PROVISIONAL APPLICATION NUMBER **FILING DATE**

_____ / _____ _____
_____ / _____ _____
_____ / _____ _____

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S) UNDER 35 U.S.C. SECTION 120

[ ] The claim for the benefit of any such applications are set forth in the attached ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART (C-I-P) APPLICATION.

ALL FOREIGN APPLICATION(S), *IF ANY*, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

_____

_____

_____

NOTE: *If the application filed more than 12 months from the filing date of this application is a PCT filing forming the basis for this application entering the United States as (1) the national stage, or (2) a continuation, divisional, or continuation-in-part, then also complete ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR C-I-P APPLICATION for benefit of the prior U.S. or PCT application(s) under 35 U.S.C. Section 120.*

POWER OF ATTORNEY

I hereby appoint the following practitioner(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

(list name and registration number)

JOSEPH H. HANDELMAN, 26179

JOHN RICHARDS, 31053

RICHARD J. STREIT, 25765

PETER D. GALLOWAY, 27885

IAN C. BAILLIE, 24090

THOMAS F. PETERSON, 24790

RICHARD P. BERG, 28145

JULIAN H. COHEN, 20302

WILLIAM R. EVANS 25858

JANET I. CORD, 33778

CLIFFORD J. MASS, 30086

CYNTHIA R. MILLER, 34678

(Check the following item, if applicable)

[ ] I hereby appoint the practitioner(s) associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

[ ] Attached, as part of this declaration and power of attorney, is the authorization of the above-named practitioner(s) to accept and follow instructions from my representative(s).

NOTE: *"Special care should be taken in continuation or divisional applications to ensure that any change of correspondence address in a prior application is reflected in the continuation or divisional application. For example, where a copy of the oath or declaration from the prior application is submitted for a continuation or divisional application filed under 37 CFR 1.53(b) and the copy of the oath or declaration from the prior application designates an old correspondence address, the Office may not recognize, in the continuation or divisional application, the change of correspondence address made during the prosecution of the prior application. Applicant is required to identify the change of correspondence address in the continuation or divisional application to ensure that communications from the Office are mailed to the current correspondence address. 37 CFR 1.63(d)(4)." Section 601.03, M.P.E.P., 7th Ed*

SEND CORRESPONDENCE TO

Ladas & Parry
26 West 61st Street
New York, N.Y. 10023

DIRECT TELEPHONE CALLS TO:
(Name and telephone number)
William R. Evans

(212) 708-1930

(complete the following if applicable)

Since this filing is a [ ] continuation [ ] divisional there is attached hereto a Change of Correspondence Address so that there will be no question as to where the PTO should direct all correspondence.

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE(S)

NOTE: Carefully indicate the family (or last) name, as it should appear on the filing receipt and all other document.

NOTE: Each inventor must be identified by full name, including the family name, and at least one given name without abbreviation together with any other given name or initial, and by his/her residence, post office address and country of citizenship. 37 C.F.R. Section 1.63(a)(3).

NOTE: Inventors may execute separate declarations/oaths provided each declaration/oath sets forth all the inventors. Section 1.63(a)(3) requires that a declaration/oath, inter alia, identify each inventor and prohibits the execution of separate declarations/oaths which each sets forth only the name of the executing inventor. 62 Fed. Reg. 53,131, 53,142, October 10, 1997.

Full name of sole or first inventor

1-00

Chong (*Given Name*) Jin (*Middle Initial or Name*) Oon (*Family (Or Last Name)*)

Inventor's signature (x) _____

Date (x) 28th February 2001. **Country of Citizenship** Singapore SGX

Residence 14A Princess of Wales Road, Singapore 266914

Post Office Address Same as above

Full name of second joint inventor, if any

2-00

Gek (*Given Name*) Keow (*Middle Initial or Name*) Lim (*Family (Or Last Name)*)

Inventor's signature (x) Keow

Date (x) 28 Feb 2001 **Country of Citizenship** Singapore SGX

Residence 16 Telok Kurau, Lorong G, Singapore 426180

Post Office Address Same as above

Full name of third joint inventor, if any

3-00

Ai (*Given Name*) Lin (*Middle Initial or Name*) Leong (*Family (Or Last Name)*)

Inventor's signature (x) _____

Date (x) 28 Feb 2001 **Country of Citizenship** Singapore SGX

Residence Block 263 Yishun Street 22 #12-161, Singapore 760263

Post Office Address Save as above

Practitioner's Docket No. U013108-9

ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR SIGNATURE BY FOURTH AND SUBSEQUENT INVENTORS

Full name of fourth joint inventor, if any

4-00

Yi (*Given Name*) | (*Middle Initial or Name*) | Zhao (*Family (Or Last Name)*)

Inventor's signature (x) Zhao

Date (x) 28 Feb 2001 **Country of Citizenship** Singapore

SGX

Residence Block 345, #12-270 Bukit Batok Street 34, Singapore 650345

Post Office Address Save as above

Full name of fifth joint inventor, if any

5-00

Wei (*Given Name*) | Ning (*Middle Initial or Name*) | Chen (*Family (Or Last Name)*)

Inventor's signature (x) [signature]

SGX

Date (x) Feb 28, 2001 **Country of Citizenship** Singapore

Residence Block 104, #22-114 Spottiswoode Park Road, Singapore 080104

Post Office Address Same as above

Full name of sixth joint inventor, if any

(*Given Name*) | (*Middle Initial or Name*) | (*Family (Or Last Name)*)

Inventor's signature _____

Date _____ **Country of Citizenship** _____

Residence _____

Post Office Address _____

(check proper box(es) for any of the following added page(s) that form a part of this declaration)

[x] **Signature** for fourth and subsequent joint inventors. *Number of pages added* __1__

* * *

[ ] **Signature** by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. *Number of pages added* _____

* * *

[ ] **Signature** for inventor who refuses to sign or cannot be reached by person authorized under 37 C.F.R. Section 1.47. *Number of pages added* _____

* * *

[ ] Added page for **signature** by one joint inventor on behalf of deceased inventor(s) where legal representative cannot be appointed in time. (37 C.F.R. Section 1.47)

* * *

[ ] Added pages to combined declaration and power of attorney for divisional, continuation, or continuation-in-part (C-I-P) application.
[ ] Number of pages added
_____

* * *

[ ] Authorization of practitioner(s) to accept and follow instructions from representative.

(If no further pages form a part of this Declaration, then end this Declaration with this page and check the following item)

[ ] This declaration ends with this page.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Oon, Chong Jin
Lim, Gek Keow
Leong, Ai Lin
Zhao, Yi
Chen, Wei Ning

(ii) TITLE OF INVENTION: A VACCINE-INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Ladas & Parry
(B) STREET: 26 West 61 Street
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: USA
(F) ZIP: 10023

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:09/719,533
(B) FILING DATE:13-DEC-2000
(C) CLASSIFICATION:435

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/SG98/00045
(B) FILING DATE: 19-JUN-1998

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Miller, Cynthia R.
(B) REGISTRATION NUMBER: 34,678
(C) REFERENCE/DOCKET NUMBER:U-013108-9

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 708-1890

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3215 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double

(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTCCACCACT TTCCACCAAA CTCTTCAAGA TCCCAGAGTC AGGGCCCTGT ACTTTCCTGC 60

TGGTGGCTCC AGTTCAGGAA CAGTGAGCCC TGCTCAGAAT ACTGTCTCTG CCATATCGTC 120

AATCTTATCG AAGACTGGGG ACCCTGTACC GAACATGGAG AACATCGCAT CAGGACTCCT 180

AGGACCCCTG CTCGTGTTAC AGGCGGGGTT TTTCTTGTTG ACAAAAATCC TCACAATACC 240

GCAGAGTCTA GACTCGTGGT GGACTTCTCT CAATTTTCTA GGGGGAACAC CCGTGTGTCT 300

TGGCCAAAAT TCGCAGTCCC AAATCTCCAG TCACTCACCA ACCTGTTGTC CTCCAATTTG 360

TCCTGGTTAT CGCTGGATGT GTCTGCGGCG TTTTATCATC TTCCTCTGCA TCCTGCTGCT 420

ATGCCTCATC TTCTTGTTGG TTCTTCTGGA CTATCAAGGT ATGTTGCCCG TTTGTCCTCT 480

AATTCCAGGA TCAACAACAA CCAGCACCGG ACCATGCAAA ACCTGCACAA CTCCTGCTCA 540

AGGAACCTCT ATGTTTCCCT CATGTTGCTG TACAAAACCT ACGGACAGAA ACTGCACCTG 600

TATTCCCATC CCATCATCTT GGGCTTTCGC AAAATACCTA TGGGAGTGGG CCTCAGTCCG 660

TTTCTCTTGG CTCAGTTTAC TAGTGCCATT TGTTCAGTGG TTCGTAGGGC TTTCCCCCAC 720

TGTCTGGCTT TCAGTTATAT GGATGATGTG GTTTTGGGGG CCAAGTCTGT ACAACATCTT 780

GAGTCCCTTT ATGCCGCTGT TACCAATTTT CTTTTGTCTT TGGGTATACA TTTAAACCCT 840

CACAAAACAA AAAGATGGGG ATATTCCCTT AACTTCATGG GATATGTCAT TGGGAGTTGG 900

GGCACATTGC CACAGGAACA TATTGTACAA AAAATCAAAA TGTGTTTTAG GAAACTTCCT 960

GTAAACAGGC CTATTGATTG GAAAGTATGT CAACGAATTG TGGGTCTTTT GGGGTTTGCC 10
20

GCCCCTTTCA CGCAATGTGG ATATCCTGCT TTAATGCCTT TATATGCATG TATACAAGCA 10
80

AAACAGGCTT TTACTTTCTC GCAAACTTAC AAGACCTTTC TAAGTAAACA GTATCTGAAC 11
40

CTTTACCCCG TTGCTCGGCA ACGCCCTGGT CTGTGCCAAG TGTTTGCTGA CGCAACCCCC 12
00

ACTGGTTGGG GCTTGGCCAT AGGCCATCAG CGCATGCGTG GAACCTTTGT GTCTCCTCTG 12
60

CCGATCCATA CTGCGGAACT CCTAGCCGCT TGTTTTGCTC GCAGCAGGTC TGGGGCAAAA 13
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CTCATCGGGA CTGACAATTC TGTCGTGCTC TCCCGCAAGT ATACATCATT TCCATGGCTG 13
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CTAGGCTGTG CTGCCAACTG GATCCTGCGC GGGACGTCCT TTGTTTACGT CCCGTCGGCG 14
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CTGAATCCCG CGGACGACCC CTCCCGGGGC CGCTTGGGGC TCTACCGCCC GCTTCTCCGC 15
00

CTGTTATACC GACCGACCAC GGGGCGCACC TCTCTTTACG CGGACTCCCC GTCTGTGCCT 15
60

TCTCATCTGC CGGACCGTGT GCACTTCGCT TCACCTCTGC ACGTCGCATG GAGACCACCG 16
20

TGAACGCCCA CGGGAACCTG CCCAAGGTCT TGCATAAGAG GACTCTTGGA CTTTCAGCAA 16
80

TGTCAACGAC CGACCTTGAG GCATACTTCA AAGACTGTGT GTTTAATGAG TGGGAGGAGT 17
40

TGGGGGAGGA GGTTAGGTTA AAGGTCTTTG TACTAGGAGG CTGTAGGCAT AAATTGGTGT 18
00

GTTCACCATC ACCATGCAAC TTTTTCACCT CTGCCTAATC ATCTCATGTT CATGTCCTAC 18
60

TGTTCAAGCC TCCAAGCTGT GCCTTGGGTG GCTTTGGGGC ATGGACATTG ACCCGTATAA 19
20

AGAATTTGGA GCTTCTGTGG AGTTACTCTC TTTTTTGCCT TCTGACTTTT TTCCTTCTAT 19
80

TCGAGATCTC CTCGACACCG CCTCTGCTCT GTATCGGGAG GCCTTAGAGT CTCCGGAACA 20

40

TTGTTCACCT CACCATACGG CACTCAGGCA AGCTATTCTG AGTTGGGGTG AGTTAATGAA 21
00

TCTAGCCACC TGGGTGGGAA GTAATTTGGA AGATCCAGCA TCCAGGGAAT TAGTAGTCAG 21
60

CTATGTCAAC GTTAATATGG GCCTAAAAAT CAGACAACTA TTGTGGTTTC ACATTTCCTG 22
20

TCTTACTTTT GGGAGAGAAA CTGTTCTTGA ATATTTGGTG TCTTTTGGAG TGTGGATTCG 22
80

CACTCCTCCT GCATATAGAC CACCAAATGC CCCTATCTTA TCAACACTTC CGGAAACTAC 23
40

TGTTGTTAGA CGAAGAGGCA GGTCCCCTAG AAGAAGAACT CCCTCGCCTC GCAGACGAAG 24
00

GTCTCAATCG CCGCGTCGCA GAAGATCTCA ATCTCGGGAA TCTCAATGTT AGTATTCCTT 24
60

GGACACATAA GGTGGGAAAC TTTACGGGGC TTTATTCTTC TACGGTACCT TGCTTTAATC 25
20

CTAAATGGCA AACTCCTTCT TTTCCGGACA TTCATTTGCA GGAGGACATT CTTGATAGAT 25
80

GTAAGCAATT TGTGGGGCCC CTTACAGTAA ATGAAAACAG GAGACTAAAA TTAATTATGC 26
40

CTGCTAGGTT TTATCCAAAT GTTACTAAAT ATTTGCCCTT AGATAAAGGG ATCAAACCAT 27
00

ATTATCCAGA GTATGTAGTT AATCATTACT TCCAGACGCG ACATTATTTA CACACTCTTT 27
60

GGAAGGCGGG GATCTTATAT AAAAGAGAGT CCACACGTAG CGCCTCATTT TGCGGGTCAC 28
20

CATATTCTTG GGAACAAGAT CTACAGCATG GGAGGTTGGT CTTCCAAACC TCGAAAAGGC 28
80

ATGGGGACAA ATCTTTCTGT CCCCAATCCC CTGGGATTCT TCCCCGATCA TCAGTTGGAC 29
40

CCTGCATTCA AAGCCAACTC AGAAAATCCA GATTGGGACC TCAACCCGCA CAAGGACAAC 30
00

TGGCCGGACG CCAACAAGGT GGGAGTGGGA GCATTCGGGC CAGGGTTCAC CCCTCCTCAT 30
60

GGGGGACTGT TGGGGTGGAG CCCTCAGGCT CAGGGCCTAC TCACAACTGT GCCAGCAGCT 31
20

CCTCCTCCTG CCTCCACCAA TCGGCAGTCA GGAAGGCAGC CTACTCCCTT ATCTCCACCT 31
80

CTAAGGGACA CTCATCCTCA GGCCATGCAG TGGAA 32
15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 843 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Pro Leu Ser Tyr Gln His Phe Arg Lys Leu Leu Leu Leu Asp Glu
1 5 10 15

Glu Ala Gly Pro Leu Glu Glu Glu Leu Pro Arg Leu Ala Asp Glu Gly
20 25 30

Leu Asn Arg Arg Val Ala Glu Asp Leu Asn Leu Gly Asn Leu Asn Val
35 40 45

Ser Ile Pro Trp Thr His Lys Val Gly Asn Phe Thr Gly Leu Tyr Ser
50 55 60

Ser Thr Val Pro Cys Phe Asn Pro Lys Trp Gln Thr Pro Ser Phe Pro
65 70 75 80

Asp Ile His Leu Gln Glu Asp Ile Leu Asp Arg Cys Lys Gln Phe Val
85 90 95

Glu Pro Leu Thr Val Asn Glu Asn Arg Arg Leu Lys Leu Ile Met Pro
100 105 110

Ala Arg Phe Tyr Pro Asn Val Thr Lys Tyr Leu Pro Leu Asp Lys Gly
115 120 125

Ile Lys Pro Tyr Tyr Pro Glu Tyr Val Val Asn His Tyr Phe Gln Thr
130 135 140

Arg His Tyr Leu His Thr Leu Trp Lys Ala Gly Ile Leu Tyr Lys Arg
145 150 155 160

Glu Ser Thr Arg Ser Ala Ser Phe Cys Gly Ser Pro Tyr Ser Trp Glu
165 170 175

Gln Asp Leu Gln His Gly Arg Leu Val Phe Gln Thr Ser Lys Arg His
180 185 190

Gly Asp Lys Ser Phe Cys Pro Glu Ser Pro Gly Ile Leu Pro Arg Ser
195 200 205

Ser Val Gly Pro Cys Ile Gln Ser Gln Leu Arg Lys Ser Arg Leu Gly
210 215 220

Pro Gln Pro Ala Gln Gly Gln Leu Ala Gly Arg Gln Gln Gly Gly Ser
225 230 235 240

Gly Ser Ile Arg Ala Arg Val His Pro Ser Ser Trp Gly Thr Val Gly
245 250 255

Val Glu Pro Ser Gly Ser Gly Pro Thr His Asn Cys Ala Ser Ser Ser
260 265 270

Ser Ser Cys Leu His Gln Ser Ala Val Arg Lys Ala Ala Tyr Ser Leu
275 280 285

Ile Ser Thr Ser Lys Gly His Ser Ser Ser Gly His Ala Val Glu Leu
290 295 300

His His Phe Pro Pro Asn Ser Ser Arg Ser Gln Ser Gln Gly Pro Val
305 310 315 320

Leu Ser Cys Trp Trp Leu Gln Phe Arg Asn Ser Glu Pro Cys Ser Glu
325 330 335

Tyr Cys Leu Cys His Ile Val Asn Leu Ile Glu Asp Trp Gly Pro Cys
340 345 350

Thr Glu His Gly Glu His Arg Ile Arg Thr Pro Arg Thr Pro Ala Arg
355 360 365

Val Thr Gly Gly Val Phe Leu Val Asp Lys Asn Pro His Asn Thr Ala
370 375 380

Glu Ser Arg Leu Val Val Asp Phe Ser Gln Phe Ser Arg Gly Asn Thr
385 390 395 400

Arg Val Ser Trp Pro Lys Phe Ala Val Pro Asn Leu Gln Ser Leu Thr
405 410 415

Asn Leu Leu Ser Ser Asn Leu Ser Trp Leu Ser Leu Asp Val Ser Ala
420 425 430

```
Ala Phe Tyr His Leu Pro Leu His Pro Ala Ala Met Pro His Leu Leu
        435                 440                 445

Val Gly Ser Ser Gly Leu Ser Arg Tyr Val Ala Arg Leu Ser Ser Asn
    450                 455                 460

Ser Arg Ile Asn Asn Asn Glu His Arg Thr Met Glu Asn Leu His Asn
465                 470                 475                 480

Ser Cys Ser Arg Asn Leu Tyr Val Ser Leu Met Leu Leu Tyr Lys Thr
                485                 490                 495

Tyr Gly Gln Lys Leu His Leu Tyr Ser His Pro Ile Ile Leu Gly Phe
            500                 505                 510

Arg Lys Ile Pro Met Gly Val Gly Leu Ser Pro Phe Leu Leu Ala Gln
        515                 520                 525

Phe Thr Ser Ala Ile Cys Ser Val Val Arg Arg Ala Phe Pro His Cys
    530                 535                 540

Leu Ala Phe Ser Tyr Met Asp Asp Val Val Leu Gly Ala Lys Ser Val
545                 550                 555                 560

Gln His Leu Glu Ser Leu Tyr Ala Ala Val Thr Asn Phe Leu Leu Ser
                565                 570                 575

Leu Gly Ile His Leu Asn Pro His Lys Thr Lys Arg Trp Gly Tyr Ser
            580                 585                 590

Leu Asn Phe Met Gly Tyr Val Ile Gly Ser Trp Gly Thr Leu Pro Gln
        595                 600                 605

Glu His Ile Val Gln Lys Ile Lys Met Cys Phe Arg Lys Leu Pro Val
    610                 615                 620

Asn Arg Pro Ile Asp Trp Lys Val Cys Gln Arg Ile Val Gly Leu Leu
625                 630                 635                 640

Gly Phe Ala Ala Pro Phe Thr Gln Cys Gly Tyr Pro Ala Leu Met Pro
                645                 650                 655

Leu Tyr Ala Cys Ile Gln Ala Lys Gln Ala Phe Thr Phe Ser Gln Thr
            660                 665                 670

Tyr Lys Thr Phe Leu Ser Lys Gln Tyr Leu Asn Leu Tyr Pro Val Ala
        675                 680                 685

Arg Gln Arg Pro Gly Leu Cys Glu Val Phe Ala Asp Ala Thr Pro Thr
    690                 695                 700
```

```
Gly Trp Gly Leu Ala Ile Gly His Gln Arg Met Arg Gly Thr Phe Val
705                 710                 715                 720

Ser Pro Leu Pro Ile His Thr Ala Glu Leu Leu Ala Ala Cys Phe Ala
                725                 730                 735

Arg Ser Arg Ser Gly Ala Lys Leu Ile Gly Thr Asp Asn Ser Val Val
            740                 745                 750

Leu Ser Arg Lys Tyr Thr Ser Phe Pro Trp Leu Leu Gly Cys Ala Ala
        755                 760                 765

Asn Trp Ile Leu Arg Gly Thr Ser Phe Val Tyr Val Pro Ser Ala Leu
    770                 775                 780

Asn Pro Ala Asp Asp Pro Ser Arg Gly Arg Leu Gly Leu Tyr Arg Pro
785                 790                 795                 800

Leu Leu Arg Leu Leu Tyr Arg Pro Thr Thr Gly Arg Thr Ser Leu Tyr
                805                 810                 815

Ala Asp Ser Pro Ser Val Pro Ser His Leu Pro Asp Arg Val His Phe
            820                 825                 830

Ala Ser Pro Leu His Val Ala Trp Arg Pro Pro
        835                 840
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 400 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Gly Gly Trp Ser Ser Lys Pro Arg Lys Gly Met Gly Thr Asn Leu
1               5                   10                  15

Ser Val Pro Asn Pro Leu Gly Phe Phe Pro Asp His Gln Leu Asp Pro
            20                  25                  30

Ala Phe Lys Ala Asn Ser Glu Asn Pro Asp Trp Asp Leu Asn Pro His
        35                  40                  45

Lys Asp Asn Trp Pro Asp Ala Asn Lys Val Gly Val Gly Ala Phe Gly
```

```
    50                  55                  60

Pro Gly Phe Thr Pro Pro His Gly Gly Leu Leu Gly Trp Ser Pro Gln
65                  70                  75                  80

Ala Gln Gly Leu Leu Thr Thr Val Pro Ala Ala Pro Pro Pro Ala Ser
                85                  90                  95

Thr Asn Arg Gln Ser Gly Arg Gln Pro Thr Pro Leu Ser Pro Pro Leu
            100                 105                 110

Arg Asp Thr His Pro Gln Ala Met Gln Trp Asn Ser Thr Thr Phe His
        115                 120                 125

Gln Thr Leu Gln Asp Pro Arg Val Arg Ala Leu Tyr Phe Pro Ala Gly
    130                 135                 140

Gly Ser Ser Ser Gly Thr Val Ser Pro Ala Gln Asn Thr Val Ser Ala
145                 150                 155                 160

Ile Ser Ser Ile Leu Ser Lys Thr Gly Asp Pro Val Pro Asn Met Glu
                165                 170                 175

Asn Ile Ala Ser Gly Leu Leu Gly Pro Leu Leu Val Leu Gln Ala Gly
            180                 185                 190

Phe Phe Leu Leu Thr Lys Ile Leu Thr Ile Pro Gln Ser Leu Asp Ser
        195                 200                 205

Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Pro Thr Val Cys Leu Gly
    210                 215                 220

Gln Asn Ser Gln Ser Gln Ile Ser Ser His Ser Pro Thr Cys Cys Pro
225                 230                 235                 240

Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile Ile
                245                 250                 255

Phe Leu Cys Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu
            260                 265                 270

Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser Thr
        275                 280                 285

Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln Gly
    290                 295                 300

Thr Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Thr Asp Arg Asn
305                 310                 315                 320

Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys Tyr Leu
                325                 330                 335
```

```
Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val Pro
            340                 345                 350

Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser Val
        355                 360                 365

Ile Trp Met Met Trp Phe Trp Gly Pro Ser Leu Tyr Asn Ile Leu Ser
    370                 375                 380

Pro Phe Met Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr Ile
385                 390                 395                 400
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 212 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Gln Leu Phe His Leu Cys Leu Ile Ile Ser Cys Ser Cys Pro Thr
1               5                  10                  15

Val Gln Ala Ser Lys Leu Cys Leu Gly Trp Leu Trp Gly Met Asp Ile
            20                  25                  30

Asp Pro Tyr Lys Glu Phe Gly Ala Ser Val Glu Leu Leu Ser Phe Leu
        35                  40                  45

Pro Ser Asp Phe Phe Pro Ser Ile Arg Asp Leu Leu Asp Thr Ala Ser
    50                  55                  60

Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys Ser Pro His
65                  70                  75                  80

His Thr Ala Leu Arg Gln Ala Ile Leu Ser Trp Gly Glu Leu Met Asn
                85                  90                  95

Leu Ala Thr Trp Val Gly Ser Asn Leu Glu Asp Pro Ala Ser Arg Glu
            100                 105                 110

Leu Val Val Ser Tyr Val Asn Val Asn Met Gly Leu Lys Ile Arg Gln
        115                 120                 125

Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg Glu Thr Val
    130                 135                 140
```

```
Leu Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr Pro Pro Ala
145                 150                 155                 160

Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro Glu Thr Thr
                165                 170                 175

Val Val Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr Pro Ser Pro
            180                 185                 190

Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser Gln Ser Arg
        195                 200                 205

Glu Ser Gln Cys
    210
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 154 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Ala Ala Arg Leu Cys Cys Gln Leu Asp Pro Ala Arg Asp Val Leu
1               5                   10                  15

Cys Leu Arg Pro Val Gly Ala Glu Ser Arg Gly Arg Pro Leu Pro Gly
            20                  25                  30

Pro Leu Gly Ala Leu Pro Pro Ala Ser Pro Pro Val Ile Pro Thr Asp
        35                  40                  45

His Gly Ala His Leu Ser Leu Arg Gly Leu Pro Val Cys Ala Phe Ser
    50                  55                  60

Ser Ala Gly Pro Cys Ala Leu Arg Phe Thr Ser Ala Arg Arg Met Glu
65                  70                  75                  80

Thr Thr Val Asn Ala His Gly Asn Leu Pro Lys Val Leu His Lys Arg
                85                  90                  95

Thr Leu Gly Leu Ser Ala Met Ser Thr Thr Asp Leu Glu Ala Tyr Phe
            100                 105                 110

Lys Asp Cys Val Phe Asn Glu Trp Glu Glu Leu Gly Glu Glu Val Arg
        115                 120                 125

Leu Lys Val Phe Val Leu Gly Gly Cys Arg His Lys Leu Val Cys Ser
```

130 135 140

Pro Ser Pro Cys Asn Phe Phe Thr Ser Ala
145 150

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATAAGCTTAT GCCCCTATCT TATCAACACT TCCGGA
36

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAGTCTAGAC TCTGCGGTAT TGTGA
25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAGTCTAGAC TCGTGGTGGA CTTCT
25

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGAGAATTCT CACGGTGGTC TCCATGCGAC GT
32

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TTTGTTTACG TCCCGT
16

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTAAGCTTAG TTTCCGGAAG TGTTGAT
27

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) TITLE OF INVENTION: A VACCINE-INDUCED HEPATITIS B VIRAL STRAIN AND USES THEREOF

(ii) NUMBER OF SEQUENCES: 11

(2) INFORMATION FOR SEQ ID NO:1: (Figure 3)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3215 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTCCACCACT TTCCACCAAA CTCTTCAAGA TCCCAGAGTC AGGGCCCTGT ACTTTCCTGC 60

TGGTGGCTCC AGTTCAGGAA CAGTGAGCCC TGCTCAGAAT ACTGTCTCTG CCATATCGTC 120

AATCTTATCG AAGACTGGGG ACCCTGTACC GAACATGGAG AACATCGCAT CAGGACTCCT 180

AGGACCCCTG CTCGTGTTAC AGGCGGGGTT TTTCTTGTTG ACAAAAATCC TCACAATACC 240

GCAGAGTCTA GACTCGTGGT GGACTTCTCT CAATTTTCTA GGGGGAACAC CCGTGTGTCT 300

TGGCCAAAAT TCGCAGTCCC AAATCTCCAG TCACTCACCA ACCTGTTGTC CTCCAATTTG 360

TCCTGGTTAT CGCTGGATGT GTCTGCGGCG TTTTATCATC TTCCTCTGCA TCCTGCTGCT 420

ATGCCTCATC TTCTTGTTGG TTCTTCTGGA CTATCAAGGT ATGTTGCCCG TTTGTCCTCT 480

AATTCCAGGA TCAACAACAA CCAGCACCGG ACCATGCAAA ACCTGCACAA CTCCTGCTCA 540

AGGAACCTCT ATGTTTCCCT CATGTTGCTG TACAAAACCT ACGGACAGAA ACTGCACCTG 600

TATTCCCATC CCATCATCTT GGGCTTTCGC AAAATACCTA TGGGAGTGGG CCTCAGTCCG 660

TTTCTCTTGG CTCAGTTTAC TAGTGCCATT TGTTCAGTGG TTCGTAGGGC TTTCCCCCAC 720

TGTCTGGCTT TCAGTTATAT GGATGATGTG GTTTTGGGGG CCAAGTCTGT ACAACATCTT 780

GAGTCCCTTT ATGCCGCTGT TACCAATTTT CTTTTGTCTT TGGGTATACA TTTAAACCCT 840

CACAAAACAA AAAGATGGGG ATATTCCCTT AACTTCATGG GATATGTCAT TGGGAGTTGG 900

GGCACATTGC CACAGGAACA TATTGTACAA AAAATCAAAA TGTGTTTTAG GAAACTTCCT 960

GTAAACAGGC CTATTGATTG GAAAGTATGT CAACGAATTG TGGGTCTTTT GGGGTTTGCC 1020

GCCCCTTTCA CGCAATGTGG ATATCCTGCT TTAATGCCTT TATATGCATG TATACAAGCA 1080

AAACAGGCTT TTACTTTCTC GCAAACTTAC AAGACCTTTC TAAGTAAACA GTATCTGAAC 1140

CTTTACCCCG TTGCTCGGCA ACGCCCTGGT CTGTGCCAAG TGTTTGCTGA CGCAACCCCC 1200

ACTGGTTGGG GCTTGGCCAT AGGCCATCAG CGCATGCGTG GAACCTTTGT GTCTCCTCTG 1260

CCGATCCATA CTGCGGAACT CCTAGCCGCT TGTTTTGCTC GCAGCAGGTC TGGGGCAAAA 1320
CTCATCGGGA CTGACAATTC TGTCGTGCTC TCCCGCAAGT ATACATCATT TCCATGGCTG 1380
CTAGGCTGTG CTGCCAACTG GATCCTGCGC GGGACGTCCT TTGTTTACGT CCCGTCGGCG 1440
CTGAATCCCG CGGACGACCC CTCCCGGGGC CGCTTGGGGC TCTACCGCCC GCTTCTCCGC 1500
CTGTTATACC GACCGACCAC GGGGCGCACC TCTCTTTACG CGGACTCCCC GTCTGTGCCT 1560
TCTCATCTGC CGGACCGTGT GCACTTCGCT TCACCTCTGC ACGTCGCATG GAGACCACCG 1620
TGAACGCCCA CGGGAACCTG CCCAAGGTCT TGCATAAGAG GACTCTTGGA CTTTCAGCAA 1680
TGTCAACGAC CGACCTTGAG GCATACTTCA AAGACTGTGT GTTTAATGAG TGGGAGGAGT 1740
TGGGGGAGGA GGTTAGGTTA AAGGTCTTTG TACTAGGAGG CTGTAGGCAT AAATTGGTGT 1800
GTTCACCATC ACCATGCAAC TTTTTCACCT CTGCCTAATC ATCTCATGTT CATGTCCTAC 1860
TGTTCAAGCC TCCAAGCTGT GCCTTGGGTG GCTTTGGGGC ATGGACATTG ACCCGTATAA 1920
AGAATTTGGA GCTTCTGTGG AGTTACTCTC TTTTTTGCCT TCTGACTTTT TTCCTTCTAT 1980
TCGAGATCTC CTCGACACCG CCTCTGCTCT GTATCGGGAG GCCTTAGAGT CTCCGGAACA 2040
TTGTTCACCT CACCATACGG CACTCAGGCA AGCTATTCTG AGTTGGGGTG AGTTAATGAA 2100
TCTAGCCACC TGGGTGGGAA GTAATTTGGA AGATCCAGCA TCCAGGGAAT TAGTAGTCAG 2160
CTATGTCAAC GTTAATATGG GCCTAAAAAT CAGACAACTA TTGTGGTTTC ACATTTCCTG 2220
TCTTACTTTT GGGAGAGAAA CTGTTCTTGA ATATTTGGTG TCTTTTGGAG TGTGGATTCG 2280
CACTCCTCCT GCATATAGAC CACCAAATGC CCCTATCTTA TCAACACTTC CGGAAACTAC 2340
TGTTGTTAGA CGAAGAGGCA GGTCCCCTAG AAGAAGAACT CCCTCGCCTC GCAGACGAAG 2400
GTCTCAATCG CCGCGTCGCA GAAGATCTCA ATCTCGGGAA TCTCAATGTT AGTATTCCTT 2460
GGACACATAA GGTGGGAAAC TTTACGGGGC TTTATTCTTC TACGGTACCT TGCTTTAATC 2520
CTAAATGGCA AACTCCTTCT TTTCCGGACA TTCATTTGCA GGAGGACATT CTTGATAGAT 2580
GTAAGCAATT TGTGGGGCCC CTTACAGTAA ATGAAAACAG GAGACTAAAA TTAATTATGC 2640
CTGCTAGGTT TTATCCAAAT GTTACTAAAT ATTTGCCCTT AGATAAAGGG ATCAAACCAT 2700
ATTATCCAGA GTATGTAGTT AATCATTACT TCCAGACGCG ACATTATTTA CACACTCTTT 2760
GGAAGGCGGG GATCTTATAT AAAAGAGAGT CCACACGTAG CGCCTCATTT TGCGGGTCAC 2820
CATATTCTTG GGAACAAGAT CTACAGCATG GGAGGTTGGT CTTCCAAACC TCGAAAAGGC 2880
ATGGGGACAA ATCTTTCTGT CCCCAATCCC CTGGGATTCT TCCCCGATCA TCAGTTGGAC 2940
CCTGCATTCA AAGCCAACTC AGAAAATCCA GATTGGGACC TCAACCCGCA CAAGGACAAC 3000
TGGCCGGACG CCAACAAGGT GGGAGTGGGA GCATTCGGGC CAGGGTTCAC CCCTCCTCAT 3060
GGGGGACTGT TGGGGTGGAG CCCTCAGGCT CAGGGCCTAC TCACAACTGT GCCAGCAGCT 3120
CCTCCTCCTG CCTCCACCAA TCGGCAGTCA GGAAGGCAGC CTACTCCCTT ATCTCCACCT 3180
CTAAGGGACA CTCATCCTCA GGCCATGCAG TGGAA 3215

(2) INFORMATION FOR SEQ ID NO:2: (Figure 4)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 843 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Pro Leu Ser Tyr Gln His Phe Arg Lys Leu Leu Leu Leu Asp Glu
1               5                  10                  15

Glu Ala Gly Pro Leu Glu Glu Glu Leu Pro Arg Leu Ala Asp Glu Gly
            20                  25                  30

Leu Asn Arg Arg Val Ala Glu Asp Leu Asn Leu Gly Asn Leu Asn Val
        35                  40                  45

Ser Ile Pro Trp Thr His Lys Val Gly Asn Phe Thr Gly Leu Tyr Ser
    50                  55                  60

Ser Thr Val Pro Cys Phe Asn Pro Lys Trp Gln Thr Pro Ser Phe Pro
65                  70                  75                  80

Asp Ile His Leu Gln Glu Asp Ile Leu Asp Arg Cys Lys Gln Phe Val
                85                  90                  95

Glu Pro Leu Thr Val Asn Glu Asn Arg Arg Leu Lys Leu Ile Met Pro
            100                 105                 110

Ala Arg Phe Tyr Pro Asn Val Thr Lys Tyr Leu Pro Leu Asp Lys Gly
        115                 120                 125

Ile Lys Pro Tyr Tyr Pro Glu Tyr Val Val Asn His Tyr Phe Gln Thr
    130                 135                 140

Arg His Tyr Leu His Thr Leu Trp Lys Ala Gly Ile Leu Tyr Lys Arg
145                 150                 155                 160

Glu Ser Thr Arg Ser Ala Ser Phe Cys Gly Ser Pro Tyr Ser Trp Glu
                165                 170                 175

Gln Asp Leu Gln His Gly Arg Leu Val Phe Gln Thr Ser Lys Arg His
            180                 185                 190

Gly Asp Lys Ser Phe Cys Pro Glu Ser Pro Gly Ile Leu Pro Arg Ser
        195                 200                 205

Ser Val Gly Pro Cys Ile Gln Ser Gln Leu Arg Lys Ser Arg Leu Gly
    210                 215                 220

Pro Gln Pro Ala Gln Gly Gln Leu Ala Gly Arg Gln Gln Gly Gly Ser
225                 230                 235                 240

Gly Ser Ile Arg Ala Arg Val His Pro Ser Ser Trp Gly Thr Val Gly
                245                 250                 255

Val Glu Pro Ser Gly Ser Gly Pro Thr His Asn Cys Ala Ser Ser Ser
            260                 265                 270

Ser Ser Cys Leu His Gln Ser Ala Val Arg Lys Ala Ala Tyr Ser Leu
        275                 280                 285
```

Ile Ser Thr Ser Lys Gly His Ser Ser Ser Gly His Ala Val Glu Leu
290 295 300

His His Phe Pro Pro Asn Ser Ser Arg Ser Gln Ser Gln Gly Pro Val
305 310 315 320

Leu Ser Cys Trp Trp Leu Gln Phe Arg Asn Ser Glu Pro Cys Ser Glu
325 330 335

Tyr Cys Leu Cys His Ile Val Asn Leu Ile Glu Asp Trp Gly Pro Cys
340 345 350

Thr Glu His Gly Glu His Arg Ile Arg Thr Pro Arg Thr Pro Ala Arg
355 360 365

Val Thr Gly Gly Val Phe Leu Val Asp Lys Asn Pro His Asn Thr Ala
370 375 380

Glu Ser Arg Leu Val Val Asp Phe Ser Gln Phe Ser Arg Gly Asn Thr
385 390 395 400

Arg Val Ser Trp Pro Lys Phe Ala Val Pro Asn Leu Gln Ser Leu Thr
405 410 415

Asn Leu Leu Ser Ser Asn Leu Ser Trp Leu Ser Leu Asp Val Ser Ala
420 425 430

Ala Phe Tyr His Leu Pro Leu His Pro Ala Ala Met Pro His Leu Leu
435 440 445

Val Gly Ser Ser Gly Leu Ser Arg Tyr Val Ala Arg Leu Ser Ser Asn
450 455 460

Ser Arg Ile Asn Asn Asn Glu His Arg Thr Met Glu Asn Leu His Asn
465 470 475 480

Ser Cys Ser Arg Asn Leu Tyr Val Ser Leu Met Leu Leu Tyr Lys Thr
485 490 495

Tyr Gly Gln Lys Leu His Leu Tyr Ser His Pro Ile Ile Leu Gly Phe
500 505 510

Arg Lys Ile Pro Met Gly Val Gly Leu Ser Pro Phe Leu Leu Ala Gln
515 520 525

Phe Thr Ser Ala Ile Cys Ser Val Val Arg Arg Ala Phe Pro His Cys
530 535 540

Leu Ala Phe Ser Tyr Met Asp Asp Val Val Leu Gly Ala Lys Ser Val
545 550 555 560

Gln His Leu Glu Ser Leu Tyr Ala Ala Val Thr Asn Phe Leu Leu Ser
565 570 575

Leu Gly Ile His Leu Asn Pro His Lys Thr Lys Arg Trp Gly Tyr Ser
580 585 590

Leu Asn Phe Met Gly Tyr Val Ile Gly Ser Trp Gly Thr Leu Pro Gln
595 600 605

Glu His Ile Val Gln Lys Ile Lys Met Cys Phe Arg Lys Leu Pro Val
610 615 620

Asn Arg Pro Ile Asp Trp Lys Val Cys Gln Arg Ile Val Gly Leu Leu
625 630 635 640

```
Gly Phe Ala Ala Pro Phe Thr Gln Cys Gly Tyr Pro Ala Leu Met Pro
                645                 650                 655

Leu Tyr Ala Cys Ile Gln Ala Lys Gln Ala Phe Thr Phe Ser Gln Thr
            660                 665                 670

Tyr Lys Thr Phe Leu Ser Lys Gln Tyr Leu Asn Leu Tyr Pro Val Ala
        675                 680                 685

Arg Gln Arg Pro Gly Leu Cys Glu Val Phe Ala Asp Ala Thr Pro Thr
    690                 695                 700

Gly Trp Gly Leu Ala Ile Gly His Gln Arg Met Arg Gly Thr Phe Val
705                 710                 715                 720

Ser Pro Leu Pro Ile His Thr Ala Glu Leu Leu Ala Ala Cys Phe Ala
                725                 730                 735

Arg Ser Arg Ser Gly Ala Lys Leu Ile Gly Thr Asp Asn Ser Val Val
            740                 745                 750

Leu Ser Arg Lys Tyr Thr Ser Phe Pro Trp Leu Leu Gly Cys Ala Ala
        755                 760                 765

Asn Trp Ile Leu Arg Gly Thr Ser Phe Val Tyr Val Pro Ser Ala Leu
    770                 775                 780

Asn Pro Ala Asp Asp Pro Ser Arg Gly Arg Leu Gly Leu Tyr Arg Pro
785                 790                 795                 800

Leu Leu Arg Leu Leu Tyr Arg Pro Thr Thr Gly Arg Thr Ser Leu Tyr
                805                 810                 815

Ala Asp Ser Pro Ser Val Pro Ser His Leu Pro Asp Arg Val His Phe
            820                 825                 830

Ala Ser Pro Leu His Val Ala Trp Arg Pro Pro
        835                 840
```

(2) INFORMATION FOR SEQ ID NO:3: (Figure 5)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 400 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Gly Gly Trp Ser Ser Lys Pro Arg Lys Gly Met Gly Thr Asn Leu
1               5                   10                  15

Ser Val Pro Asn Pro Leu Gly Phe Phe Pro Asp His Gln Leu Asp Pro
            20                  25                  30

Ala Phe Lys Ala Asn Ser Glu Asn Pro Asp Trp Asp Leu Asn Pro His
        35                  40                  45

Lys Asp Asn Trp Pro Asp Ala Asn Lys Val Gly Val Gly Ala Phe Gly
    50                  55                  60

Pro Gly Phe Thr Pro Pro His Gly Gly Leu Leu Gly Trp Ser Pro Gln
65                  70                  75                  80
```

```
Ala Gln Gly Leu Leu Thr Thr Val Pro Ala Ala Pro Pro Pro Ala Ser
                85                  90                  95

Thr Asn Arg Gln Ser Gly Arg Gln Pro Thr Pro Leu Ser Pro Pro Leu
            100                 105                 110

Arg Asp Thr His Pro Gln Ala Met Gln Trp Asn Ser Thr Thr Phe His
        115                 120                 125

Gln Thr Leu Gln Asp Pro Arg Val Arg Ala Leu Tyr Phe Pro Ala Gly
    130                 135                 140
Gly Ser Ser Ser Gly Thr Val Ser Pro Ala Gln Asn Thr Val Ser Ala
145                 150                 155                 160

Ile Ser Ser Ile Leu Ser Lys Thr Gly Asp Pro Val Pro Asn Met Glu
                165                 170                 175

Asn Ile Ala Ser Gly Leu Leu Gly Pro Leu Leu Val Leu Gln Ala Gly
            180                 185                 190

Phe Phe Leu Leu Thr Lys Ile Leu Thr Ile Pro Gln Ser Leu Asp Ser
        195                 200                 205

Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Pro Thr Val Cys Leu Gly
    210                 215                 220

Gln Asn Ser Gln Ser Gln Ile Ser Ser His Ser Pro Thr Cys Cys Pro
225                 230                 235                 240

Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile Ile
                245                 250                 255

Phe Leu Cys Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu
            260                 265                 270

Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser Thr
        275                 280                 285

Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Thr Pro Ala Gln Gly
    290                 295                 300

Thr Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Thr Asp Arg Asn
305                 310                 315                 320

Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Lys Tyr Leu
                325                 330                 335

Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val Pro
            340                 345                 350

Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser Val
        355                 360                 365

Ile Trp Met Met Trp Phe Trp Gly Pro Ser Leu Tyr Asn Ile Leu Ser
    370                 375                 380

Pro Phe Met Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr Ile
385                 390                 395                 400
```

(2) INFORMATION FOR SEQ ID NO:4: (Figure 6)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 212 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Gln Leu Phe His Leu Cys Leu Ile Ile Ser Cys Ser Cys Pro Thr
1               5                  10                  15

Val Gln Ala Ser Lys Leu Cys Leu Gly Trp Leu Trp Gly Met Asp Ile
            20                  25                  30

Asp Pro Tyr Lys Glu Phe Gly Ala Ser Val Glu Leu Leu Ser Phe Leu
        35                  40                  45

Pro Ser Asp Phe Phe Pro Ser Ile Arg Asp Leu Leu Asp Thr Ala Ser
    50                  55                  60

Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys Ser Pro His
65                  70                  75                  80

His Thr Ala Leu Arg Gln Ala Ile Leu Ser Trp Gly Glu Leu Met Asn
                85                  90                  95

Leu Ala Thr Trp Val Gly Ser Asn Leu Glu Asp Pro Ala Ser Arg Glu
            100                 105                 110

Leu Val Val Ser Tyr Val Asn Val Asn Met Gly Leu Lys Ile Arg Gln
        115                 120                 125

Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg Glu Thr Val
    130                 135                 140

Leu Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr Pro Pro Ala
145                 150                 155                 160

Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro Glu Thr Thr
                165                 170                 175

Val Val Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr Pro Ser Pro
            180                 185                 190

Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser Gln Ser Arg
        195                 200                 205

Glu Ser Gln Cys
    210
```

(2) INFORMATION FOR SEQ ID NO:5: (Figure 7)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 154 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Ala Ala Arg Leu Cys Cys Gln Leu Asp Pro Ala Arg Asp Val Leu
1               5                  10                  15

Cys Leu Arg Pro Val Gly Ala Glu Ser Arg Gly Arg Pro Leu Pro Gly
            20                  25                  30

Pro Leu Gly Ala Leu Pro Pro Ala Ser Pro Pro Val Ile Pro Thr Asp
        35                  40                  45

His Gly Ala His Leu Ser Leu Arg Gly Leu Pro Val Cys Ala Phe Ser
    50                  55                  60

Ser Ala Gly Pro Cys Ala Leu Arg Phe Thr Ser Ala Arg Arg Met Glu
65                  70                  75                  80

Thr Thr Val Asn Ala His Gly Asn Leu Pro Lys Val Leu His Lys Arg
                85                  90                  95

Thr Leu Gly Leu Ser Ala Met Ser Thr Thr Asp Leu Glu Ala Tyr Phe
            100                 105                 110

Lys Asp Cys Val Phe Asn Glu Trp Glu Glu Leu Gly Glu Glu Val Arg
        115                 120                 125

Leu Lys Val Phe Val Leu Gly Gly Cys Arg His Lys Leu Val Cys Ser
    130                 135                 140

Pro Ser Pro Cys Asn Phe Phe Thr Ser Ala
145                 150
```

(2) INFORMATION FOR SEQ ID NO:6: (Figure 8)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATAAGCTTATG CCCCTATCTT ATCAACACTT CCGGA 35

(2) INFORMATION FOR SEQ ID NO:7: (Figure 9)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAGTCTAGAC TCTGCGGTAT TGTGA 25

(2) INFORMATION FOR SEQ ID NO:8: (Figure 10)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAGTCTAGAC TCGTGGTGGA CTTCT 25

(2) INFORMATION FOR SEQ ID NO:9: (Figure 11)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGAGAATTCT CACGGTGGTC TCCATGCGAC GT 32

(2) INFORMATION FOR SEQ ID NO:10: (Figure 12)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TTTGTTTACG TCCCGT 16

(2) INFORMATION FOR SEQ ID NO:11: (Figure 13)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTAAGCTTAG TTTCCGGAAG TGTTGAT 27

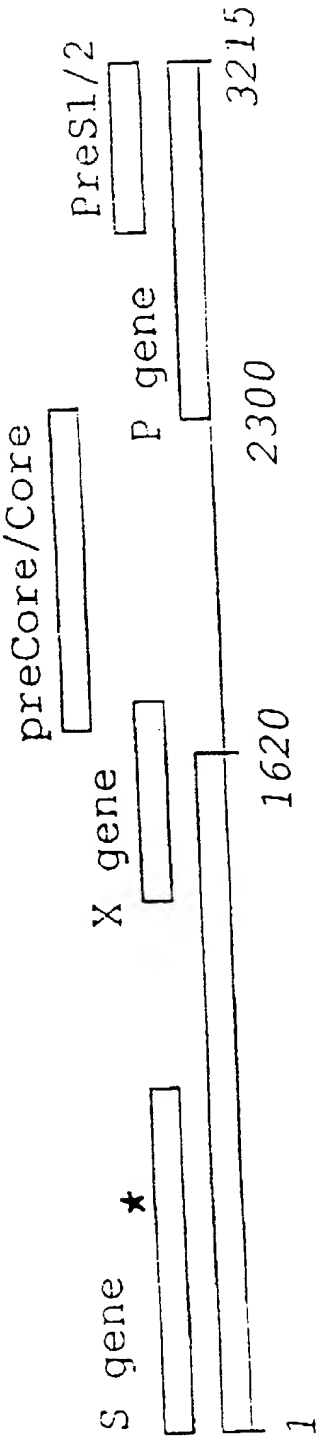
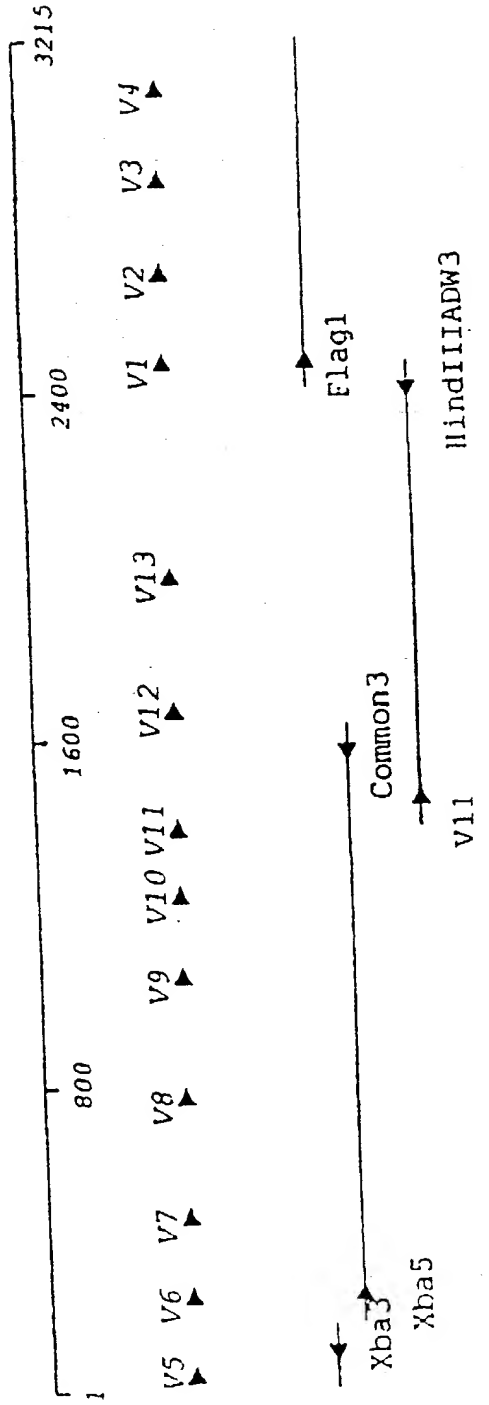


Figure 1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800
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V1
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V12
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V10
V9
V8
V7
V6
V5
Flag1
HindIIIADW3
Common3
V11
Xba3
Xba5

Figure 2